CYTOLOGICAL STUDIES OF INTERGENERIC AND INTERSPECIFIC HYBRIDS WITHIN THE GRASS TRIBE HORDEAE

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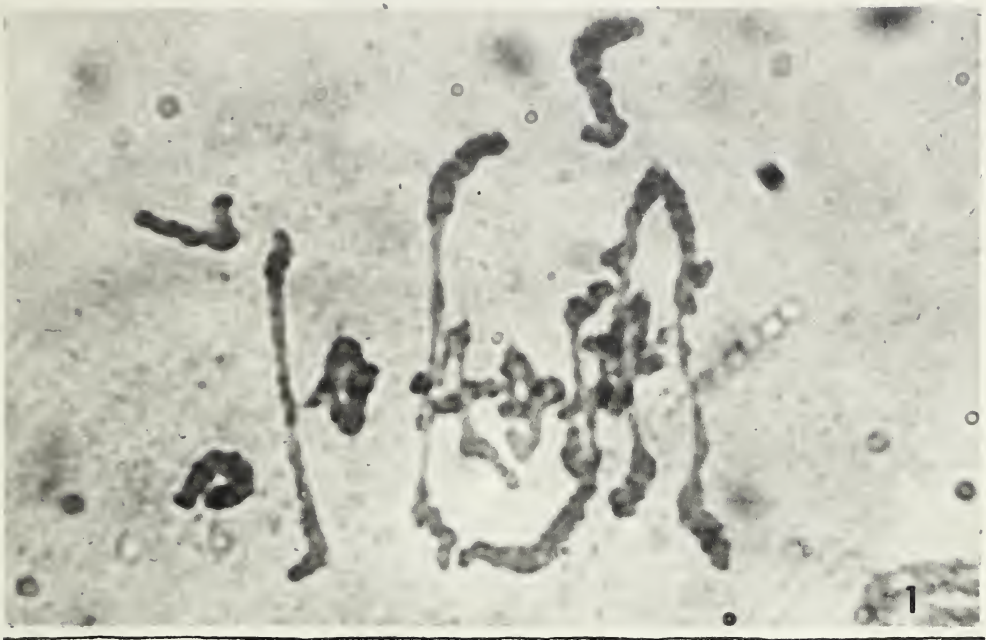
Plate I

Fig. 1. Metaphase I of hybrid Hordeum jubatum x Secale cereale var. Sangaste. Hordeum chromosomes formed autosyndetically 6 bivalents. Three rye chromosomes (shaded with R) show a trivalent configuration with one chromosome attached to it by a very thin strand. One bivalent is formed by a rye and Hordeum chromosome. The univalents consist of one Hordeum and two rye chromosomes.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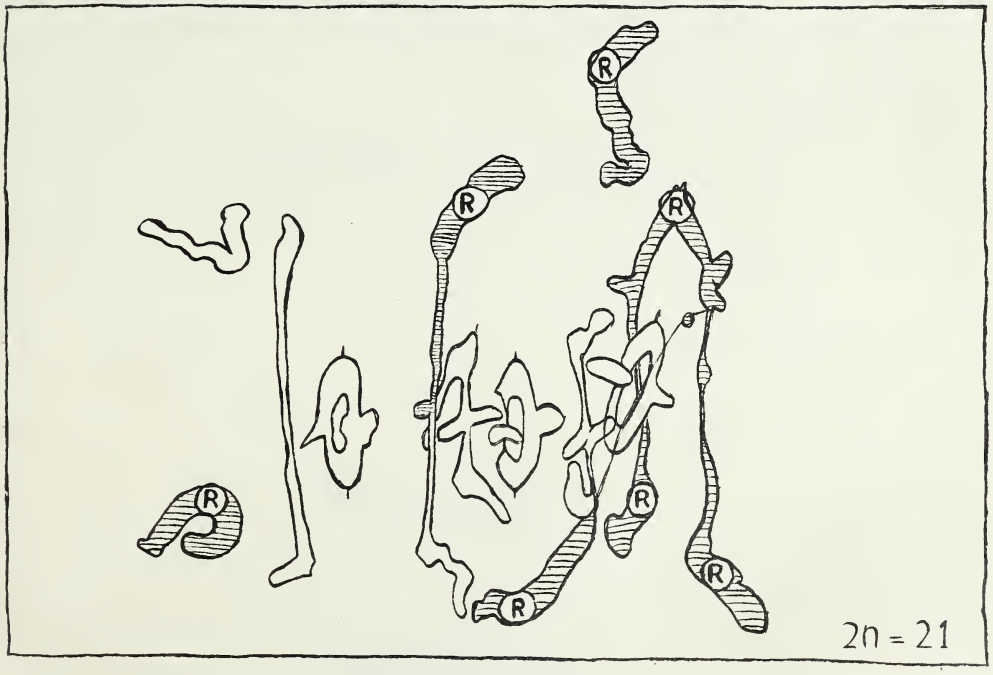
R
R
R
R
R
R
R
R
2n = 21

THE UNIVERSITY OF ALBERTA

CYTOLOGICAL STUDIES OF INTERGENERIC AND INTERSPECIFIC HYBRIDS WITHIN THE GRASS TRIBE HORDEAE

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ABSTRACT

CYTOLOGICAL STUDIES OF INTERGENERIC AND INTERSPECIFIC HYBRIDS WITHIN THE GRASS TRIBE *HORDEAE*

PART I

THE INTERGENERIC HYBRIDS BETWEEN *HORDEUM JUBATUM* L. AND *SECALE CEREALE* L.

Two hybrids of the cross *H. jubatum* (2n = 28) x *S. cereale* (2n = 14), one involving var. Antelope and the other var. Sangaste as the rye parent, were reared by embryo culture on an artificial nutrient medium. Both hybrids had a somatic number of 21 chromosomes. In meioses the rye chromosomes could be distinguished, being larger than the *H. jubatum* chromosomes. It is thus possible to observe that the *H. jubatum* chromosomes showed fairly strong autosyndesis. Average numbers of associations were 5.9 bivalents and 9.2 univalents. The *S. cereale* chromosomes were mostly observed as univalents. However, some autosyndetic bivalent and multivalent associations, and some associations with *H. jubatum* chromosomes were regularly seen in the hybrid having var. Sangaste as the rye parent.

The fairly strong autosyndesis displayed by the *H. jubatum* chromosomes is probably due to two very closely related genomes possessed by the tetraploid *H. jubatum*. It is probable, therefore, that *H. jubatum* is a segmental allotetraploid.

PART II

CYTOLOGICAL STUDIES

ON THE G-GENOME OF TRITICUM TIMOPHEEVI ZHUK.

T. timopheevi (2n = 28, genome AG) was crossed with two tetraploid wheat species of the Emmer group, i.e., T. durum (2n = 28, AB) and T. dicoccoides (2n = 28, AB). The hybrids showed very similar irregularities in meiosis (average 0.9 trivalents, 9.0 bivalents and 7.3 univalents). They showed an average seed set of 4.0%. Statistical analysis on the observed frequencies of metaphase I and anaphase I cell types indicated random lagging and polar distribution of univalents at anaphase I. This analysis further indicated that 7.5% of all egg cells theoretically should have had viable chromosome associations. The observed 4% of actually functional female gametes thus showed a discrepancy of 3.5%. This was explained on the basis of inviability of certain gametes genically unbalanced because of structural dissimilarities between some chromosomes of the B- and G-genome. The proportion of functional female gametes giving the observed seed set was, however, considered to be high enough to permit assumption of a close relationship between the two genomes in question.

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GENERAL INTRODUCTION

In the search for relationships among plant species to discover or explain evolutionary trends, hybridization is frequently employed. Crossability is considered an indication of close relationship, and genome analysis, chromosome morphology and plant morphology may give additional information. Moreover, hybridization is of great importance in the practical science of plant breeding. New gene combinations may be achieved which may prove invaluable with the production of new varieties with higher yields, greater disease resistance, cold resistance, and other useful characteristics.

In such hybridizations certain techniques have been employed to increase the chance of obtaining hybrids. An important development was the embryo culture technique. Wide crosses, naturally sterile, could now be attempted with some chance of success.

In the following dissertation, hybridization experiments will be discussed with the view of finding relationships indicating natural affinities. The experiments can be divided into two parts:

1. Hybridization of species and genera which are normally intersterile. Embryo culture was therefore employed to obtain hybrids. Table 1 shows the several crosses attempted, some of which gave hopeful indications. Others, although ovary stimulation occurred, did not show any evidence of embryo growth or germination. A good example is the cross Hordeum vulgare (2n = 14) x Secale cereale (2n = 14) which did not result in a single hybrid, although a great number of crosses were made and stimulated ovaries obtained.

Table 1. Interspecific and intergeneric crosses attempted from which hybrid embryos have been cultured in an artificial nutrient medium.

Crosses	No. of cultured embryos	Embryos germinated	Hybrid plants obtained
Hordeum jubatum x *Secale cereale*	about 350	11	4
H. jubatum x *S. cereale* (4x)	12	?	?
H. jubatum x *H. vulgare*	25	-	-
H. jubatum x *H. bulbosum*	10	1	1
H. bulbosum x *S. cereale*	69	-	-
H. bulbosum x *H. vulgare*	20	-	-
H. vulgare x *H. bulbosum*	2	1	1
H. vulgare (2x) x *S. cereale*	400	-	-
H. vulgare (4x) x *S. cereale*	150	-	-
H. vulgare (4x) x *H. bulbosum*	2	-	-
H. leporinum x *S. cereale*	26	2	-
Aegilops ventricosa x *S. cereale*	4	1	-
Ae. squarrosa x *S. cereale*	5	-	-
Elymus canadensis x *S. cereale*	10	2	1
Triticum aestivum x *H. vulgare*	3	1	-
S. cereale x *Lolium perenne*	16	-	-
Agropyron elongatum (2x) x *S. cereale*	4	-	-

Four kinds of hybrids have been produced so far:

a. Hordeum jubatum (2n = 28) x Secale cereale (2n = 14)

From this cross four hybrids have been obtained. Part I of this thesis gives a cytological description of these hybrids.

b. H. jubatum (2n = 28) x H. bulbosum (2n = 28)

This hybrid, potted in May, 1957, showed a very slow initial growth. Now it is growing more vigorously, although it has so far produced only a few heads. Some study of P.M.C's has been made and these observations are briefly discussed on pages 32 and 33.

c. H. vulgare (2n = 14) x H. bulbosum (2n = 28)

This hybrid has been only recently derived (January, 1958). It shows a very slow growth.

d. Elymus canadensis (2n = 28) x S. cereale (2n = 14)

This hybrid was obtained in June, 1957, and showed, similarly, a very slow initial growth. Now it is tillering profusely although it has not yet produced heads.

2. To carry out an investigation of the actual nature of the G-genome in Triticum timopheevi (2n = 28). T. timopheevi possesses genome A and G. Not much is known of the G-genome, since very little work has been done on it. A part of the investigation of this problem will be discussed in Part II, for which the following

crosses have been made:

Triticum durum (2n = 28) x *T. timopheevi* (2n = 28)

T. timopheevi (2n = 28) x *T. dicoccoides* (2n = 28)

These crosses usually set seed fairly easily and no embryo culturing has been employed.

PART I

THE INTERGENERIC HYBRIDS BETWEEN *HORDEUM JUBATUM* L. AND *SECALE CEREALE* L.

A. INTRODUCTION

Efforts to produce hybrids between *Hordeum jubatum* and *Secale cereale* resulted in four F_1 plants. The purpose of performing this cross was twofold:

1. To carry out an investigation of the cytological behaviour of the chromosomes in the meiosis of the F_1. Brink, Cooper and Ausherman (1944) had previously produced such a hybrid*. Their interest, however, was mainly centered on the development of the hybrid seed (Cooper and Brink, 1944; Brink and Cooper, 1944). Considering the extensive investigation they made on this matter there is little need for further studies of this kind. Although they published a separate article on the morphology and cytology of the hybrid (Brink, Cooper and Ausherman, 1944), they gave only a vague description of the chromosome behaviour at meiosis. A more thorough investigation may lead towards a better understanding of the genome homologies within or between the parental species, their relation and perhaps something on the origin of *H. jubatum*. These aspects are investigated in the present study and will be the main subject of Part I of this thesis.

2. To carry out an investigation of the possible importance of the hybrids as a forage crop. *H. jubatum* has no practical value as forage

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* This hybrid was accidentally lost just after their published investigations (personal communication).

crop, although it has some very desirable features such as frost and alkali resistance. If these features could be combined with other desirable characteristics from another species, the resulting hybridization might lead to a new, important forage crop, particularly suitable for the saline soils in the irrigated area of southern Alberta. It is hoped, therefore, that with the production of this hybrid between Foxtail (or Squirreltail) barley and rye, a step has been made towards the realization of this ambitious project.

B. MATERIALS AND METHODS

1. Plant sources

In the hybridization work two strains of H. jubatum were used: one collected in Edmonton (E) and one collected by Mr. E.R. Kerber in Saskatoon (S). This last strain was included to compare its cytological features with the other strain. This appeared necessary in connection with the H. jubatum x H. vulgare hybrid obtained by Kerber (see discussion).

As pollen parents, four rye varieties were employed: the fall varieties, Antelope, Petkus, Sangaste, and the spring variety, Prolific. These varieties were obtained from the available stock at Parkland Farm.

2. Embryo culture

Two techniques of transplanting the embryo on the surface of the nutrient medium were employed:

(1) In the first method the seeds were given a transverse slit just above the embryo with a small sharp knife. The seed coat was subsequently peeled off with a pair of forceps, and the embryo lifted out and put on the agar surface. About 50 embryos were cultured by this method; none of them showed any sign of growth or germination.

(2) Later the technique was modified as follows: The entire tip of the seed, containing the embryo, was carefully cut off, and submerged in calcium hypochlorite solution (Wall, 1954) for about two minutes; thereafter the seed tip was rinsed in sterile water, placed on the

surface of the agar and incubated at room temperature, high humidity and in weak daylight.

This last technique has been found to be very simple, quick and satisfactory. It seems that the embryo still gets full benefit of the nutrients in the medium, enabling it to develop to maturity. Another advantage of this method is that the embryo is less exposed to damage during the transfer to the medium. Also contamination is found to be very limited, especially if the hybridization is done under greenhouse conditions.

It seems that for this kind of cross the greenhouse environment is preferable. There is not only less chance of contamination of the cultures, but it appeared that seed set was also much greater than under field conditions.

When a hybrid seedling reached the height of about three inches, it was potted in sieved soil and placed under a bell jar to maintain a high humidity. It was transferred to the greenhouse after showing definite signs of having established itself.

The initial medium was made up as indicated by Brink, Cooper and Ausherman (1944). Later this was changed into the commercial Difco orchid agar, which proved to be well suited for this work. The procedure of making the medium up was consequently speeded up considerably.

The Difco orchid agar contains only inorganic salts. Some organic compounds were therefore added, as follows:

1. As a source of the vitamin B complex a yeast extract was employed

(White, 1934; Brink, Cooper and Ausherman, 1944); this was replaced by the commercial Difco yeast extract (0.04 gr./l.) (Roberts, as quoted by Street).

2. Casein hydrolosate, an amino acid complex, shown to have a stimulative effect on the postgerminal development of the immature embryo (Kent and Brink, 1947; Ziebur, Brink, Graf and Stahman, 1950; Rappaport, 1954; Harris, 1956) was added to the medium at a concentration of 1 gr./l.

3. A small amount ot tryptophan (Sanders and Burkholder, 1948) was also included in the medium at a concentration of 0.04 gr./l.

As culture containers, test tubes were very satisfactory, for grasses in particular. Some trouble, however, was encountered with the absorbent cotton plugs originally employed. In the very high humidity of the incubator the medium often became contaminated during the two- to three-month germination period often required, causing loss of several promising hybrids. Later, the cotton wool plugs were covered, after autoclaving, with thin, sterile plastic ("Saran-wrap"), which allows a certain amount of air exchange. Although less, contamination still occurred. The solution to this problem appeared to be test tubes with screw caps, which can be autoclaved in their entirety.

3. Colchicine treatment

A total of 32 hybrid clones were treated with colchicine according to the method of Sears (1941). Crowns of clones having several young tillers were wrapped with cotton wool saturated with

0.5% colchicine (15 plants), 0.75% colchicine (10 plants) and 1% colchicine (7 plants). During the treatments the plants were kept under high relative humidity, which proved reasonably effective in preventing evaporation. Each treatment lasted 5 - 6 days.

4. Cytology

Immature heads in P.M.C. stage were fixed in Carnoy's solution (1 acetic acid : 3 chloroform : 6 alcohol).

Slides were prepared according to the acetocarmine smear technique (Smith, 1947). Slides were made permanent by running through the series: 45% acetic acid, 95% alcohol, 100% alcohol, Canada balsam.

The microscope employed is a Reichert binocular with achromatic objectives. It has a camera attachment. Photographs were taken from the freshly prepared slides before they were made permanent.

C. RESULTS

Cross pollination results in fairly high fertilization percentage. This was observed by Quincke (1940) and Brink and Cooper (1944a), and confirmed by the present author. A fertilization of ten or more ovules in a head of 40 - 50 H. jubatum spikelets was not uncommon. In its initial growth the caryopsis is almost normal, but is somewhat retarded in its later development. The endosperm never becomes solid. The content of the seed is mostly watery, sometimes being completely empty.

Brink, Cooper and Ausherman (1944) obtained one hybrid plant from 81 cultured embryos of which 47 were contaminated. The author obtained four hybrid plants from about 350 cultured embryos. It should be emphasized, however, that in all a total of eleven hybrid embryos germinated of which only four reached maturity. Of the remaining seven embryos three produced coleoptiles but no roots, one only roots but no coleoptile, and three young seedlings were lost by late contamination.

The four hybrids were obtained from the following crosses:

1. H. jubatum (E) x Antelope rye (no. 33 - 56)
2. H. jubatum (E) x Sangaste rye (no. 134 - 57)

3./4. H. jubatum (S) x Prolific rye (no. 660 - 57 and 740 - 57)

Description of hybrid

A common feature of these hybrids is that the initial growth is very slow. In sufficient light they soon tiller profusely.

Table II shows the morphological characteristics of the hybrids compared with those of their parental species. Some characteristics of the hybrid are similar to those of H. jubatum (such as habit, scabrous surface), but more are similar to those of S. cereale (such as glaucous surface, auricles, sessile spikelet, membranous glume margin). The majority of the morphological features are intermediate between those of the parents (such as culm length and diameter, spike length, rachis disarticulating, glume length and width, glume awn length, etc.). Two connected features, however, the lengths of sheaths and leaf-blades, show a transgressive effect, since both appeared smaller than in the smallest parent, H. jubatum. Culm lengths, in contrast, are intermediate. As a result of these features in the hybrid, a smaller portion of the stem is covered by the sheaths than in the parents.

The spikes appear superficially very similar to those of the rye parent (Figs. 2 and 3). A closer examination of the spikelets, however, reveals that the floral organs are more or less intermediate in size and structure to those of the parental species. The spikelets consist mostly of two florets (Fig. 4c), although one floret per spikelet is not uncommon (Fig. 4b). A third floret, as rye sometimes shows, (Fig. 4a) occurs only rarely. Usually there are two glumes per spikelet in the hybrid spike; some extra glumes were rarely observed between the two florets of the hybrid spikelet. The basal hairs, characteristic of rye, were entirely lacking in the hybrid.

Fertility

The hybrids appeared to be completely sterile as was that reported by Brink, et al. (1944). The locules of mature anthers

Table II. Morphological features of Hordeum jubatum, Secale cereale and their hybrid

		H. jubatum	S. cereale	F_1 hybrid
Habit		tufted perennial	biennial	tufted perennial
Culms	growth	erect or decumbent at base	erect	erect
	diameter	1 mm.	2 - 4 mm.	1½ mm.
	height	30 - 40 cm.	110 - 130 cm.	60 - 80 cm.
	surface	not glaucous or slightly so	glaucous	glaucous
Leaves	sheath length	9 - 14 cm.	18 - 25 cm.	6 - 9 cm.
	auricles	none	small	small
	blade length	15 - 20 cm.	18 - 22 cm.	12 - 14 cm.
	blade width	3 - 4 mm.	12 - 15 mm.	3 - 3½ mm.
	surface	scabrous not glaucous or slightly so	glabrous glaucous	scabrous glaucous
Spikes	habit	nodding	mostly erect	erect
	length	6 - 8 cm.	12 - 15 cm.	8 - 10 cm.
	rachis	disarticulating	non-disarticulating	semi-disarticulating
Spikelets	no. per node	3	1	1
	attachment	outer 2 pedicellate, middle sessile	sessile	sessile
	no. of florets	1	2 - 3	1 - 2
	floret fertility	outer 2 sterile, middle fertile	2 - 3 fertile	sterile
	glume length	60 - 70 mm. (glume + awn)	8 - 10 mm.	5 mm.
	glume margin	non-membranous, slightly hispid	membranous	membranous
	glume width	very narrow	1½ - 2 mm.	¼ - ½ mm.
	glume awn	60 - 70 mm. (glume + awn)	2 - 5 mm.	20 - 25 mm.
Florets	lemma length	5 - 6 mm.	12 - 13 mm.	7 - 8 mm.
	lemma width	2 mm.	5 mm.	3 mm.
	lemma nerves	5	5	5
	lemma surface	not glaucous glabrous	glaucous middle nerve hispid	glaucous glabrous
	lemma form	flattened	keeled	rounded
	lemma awn length	5 - 6 cm.	appr. 1 cm.	appr. 2 cm.
	lemma surface of awn	finely hispid	hispid	hispid
	palea length	5 mm.	10 - 12 mm.	6 - 7 mm.
	palea width	½ mm.	2 mm.	1 mm.

smeared in acetocarmine contained only the wrinkled cell walls of dead, empty pollen grains. Since fertility is essential for a forage crop breeding program, doubling of the chromosome number of the hybrids was attempted on 30 clones. However, all attempts failed. After colchicine treatment, tillers showed the characteristic swelling at the base. They continued to grow, although they often remained much smaller in size than the controls. The heads did not set any seed. After flowering most of the treated plants died; only four plants survived the treatments.

Cytology

At metaphase I in meiosis in H. jubatum (2n = 28) 14 bivalents were always observed. The bivalents were mostly closed. Open or rod bivalents appeared in a low frequency. Trivalents and quadrivalents were never seen (Fig. 5).

In S. cereale seven usually closed bivalents were regularly formed (Fig. 6). The chromosomes are much larger than those of H. jubatum (compare Fig. 5 and 6).

The hybrid between these two species possesses 21 chromosomes (Fig. 7 - 13), as expected. This chromosome number was also observed in the hybrid obtained by Brink et al. (1944a). In their description of meiosis in the hybrid they stated that the S. cereale and H. jubatum chromosomes are ". . . nearly alike in size and form," an observation which cannot be confirmed by the present author. The rye and H. jubatum chromosomes show definite differences in size: the rye chromosomes are easily distinguishable (by their larger size) from the Hordeum chromosomes in almost every cell (Fig. 7 - 13).

Since Brink and his co-workers did not discover the size difference, they could not establish the real nature of the chromosome pairing they observed at meiosis. In the present hybrid, however, it is possible to determine exactly the affinities between Hordeum and Secale chromosomes or among themselves.

In the following pages the chromosome behaviour in the subsequent meiotic stages will be described and discussed. Only two hybrids (33 - 56 and 134 - 57) were cytologically investigated; the other two had not flowered by the time this thesis was written. Since some differences in chromosome behaviour at meiosis in these two hybrids were encountered it will be indicated to which hybrid the description refers.

Cells in the meiotic-prophase stages were not studied, since, owing to crowding of the small nucleus with chromosome material, interpretation of the cells was very difficult. This was also the case to a great extent in the very few diakinesis cells obtained. Because of these difficulties, the study of chromosome pairing was restricted to cells at metaphase I.

Metaphase I

In metaphase I the chromosomes of hybrid 33 - 56 showed mostly 6 bivalents and 9 univalents (Fig. 7 and 10). However, cells with other numbers of bivalents and univalents were frequently observed, as indicated in Table III and Fig. 7 - 13. The bivalents were nearly always composed of small chromosomes and the larger chromosomes were almost always observed as univalents. This indicates that pairing and

Table III. Chromosome associations at metaphase I of meiosis of the hybrid no. 33-56 between H. jubatum (E) and S. cereale var. Antelope

1			2	3	4	5	6	7
Chromosome associations I	II H	II S	III [1]	No. of cells	Cells %	Open bivalents	Closed bivalents	Chiasma per bivalent	Obs. X-ta per cell / 14 [2]
7	7			40	24.0	2.59	4.41	1.62	0.82
9	6			78	46.7	2.49	3.51	1.59	0.68
11	5			30	18.9	2.17	2.83	1.57	0.56
13	4			9	5.4	1.57	2.43	1.61	0.46
15	3			1	0.6				
10	4		1	3	1.8				
13	3		1 (22 chr.)	1	0.6				
5	7	1		1	0.6				
7	6	1		1	0.6				
9	5	1		1	0.6				
11	4	1		2	1.2				
			Total	167	100%	Av. 2.40	Av. 3.53	Av. 1.60	

1) H = Hordeum chromosomes; S = Secale chromosomes

2) 14 is taken as the number of chiasmata per cell for perfect bivalent formation.

bivalent formation occurred almost exclusively between the H. jubatum chromosomes themselves (autosyndesis). The large rye chromosomes usually appeared as univalents.

In cells with seven bivalents and seven univalents all of the Hordeum chromosomes were involved in the bivalents formed; all of the univalents were rye chromosomes apparently randomly scattered in the cytoplasm (Fig. 8, 9). Cells with less than seven Hordeum bivalents also showed Hordeum chromosomes among the univalents, easily distinguishable by their smaller sizes from the larger rye univalents (Fig. 7, 10, 11, 13).

In Table III, chromosome association observed in 167 cells of hybrid 33-56 are listed. Cells with five, six and seven bivalents were most frequently recorded. The averages are 5.9 bivalents, and 9.1 univalents. The frequencies of open and closed bivalents were also recorded. From these the average number of chiasmata per bivalent was calculated (columns 4, 5, 6). Since it was impossible to make observations of chiasma formation in pachytene and diakenesis, chiasma frequencies per bivalent were based on the observed bivalents in metaphase I: rod bivalents were assumed to have one chiasma, and closed or ring bivalents two. The values thus obtained (col. 6) give an indication of chiasma formation during pairing of the H. jubatum chromosomes in the early meiotic prophase. As may be seen, chiasma formation appears very constant for the four main cell types, all values being very close to the mean of 1.60 chiasmata per bivalent. This means that chiasma formation is independent of the number of bivalents formed, and that the chiasma-frequency at metaphase I is the

same for each bivalent. The average value for all cells is thus a good estimate and valid for each observed cell type. This constant chiasmata-per-bivalent value was also observed in other hybrids (Part II) and appears to be specific for each plant.

Occasionally trivalents are formed also of H. jubatum chromosomes (Fig. 15). This has been seen in four cells of which one had 22 chromosomes, the extra chromosome being from H. jubatum. In this last cell the extra chromosome probably caused the trivalent. The other three cells had the normal number of 21 chromosomes per cell. Trivalent formation, although not very frequent, thus seems possible among H. jubatum chromosomes in the haploid condition.

Occasionally the rye chromosomes form a long, loose bivalent (Fig. 12 and 13). Such a bivalent was seen in 5 of the total 167 cells recorded (Table III). It is interesting to note that this is a frequency of one rye bivalent per 31 cells, exactly the same frequency as Nordenskiöld (1939) found in a haploid rye plant.

The Secale and Hordeum univalents often showed "end-to-end" (e - e) associations following the terminology of Levan (1939, 1942), Person (1955) and others, in which the ends of two chromosomes are connected (Fig. 7, 10, 11). Walters (1954) assumed that these chromosomes are connected by matrix bands. "Side-by-side" (s - s) associations (Person, 1955) were also observed, although much less frequently (Fig. 11, 13). E - e and s - s associations between barley and rye chromosomes were very occasionally seen (Fig. 16).

The other hybrid (No. 134-57, rye parent Sangaste) showed a different behaviour of the haploid group of rye chromosomes in the hybrid. These chromosomes formed bivalents and trivalents much more frequently and occasionally they formed quadrivalents (Fig. 1, 14). Both rye and barley chromosomes appeared to be long and stretched. This feature made it very difficult to study these cells. Table IV shows the results of an analysis of ten cells which have been counted and analyzed. This table shows the same behaviour of the Hordeum chromosomes (autosyndesis) in this hybrid as that previously found in hybrid 33-56. The rye chromosomes regularly form one or, less often, two long rod bivalents, usually without a definite chiasma knob. Trivalents are also formed. In Figure 1 (front piece) a trivalent of rye chromosomes shows a long thin connection with a fourth rye chromosome. Occasionally Hordeum and Secale chromosomes form a bivalent; such a bivalent can be seen in Fig. 1, and shows a definite chiasma knob.

Table IV. Chromosome associations at metaphase I of meiosis of the hybrid (no. 134-57) between H. jubatum and S. cereale var. Sangaste

Chromosome associations						
I		II			III	No. of cells
H[1]	S[2]	H/H	H/S	S/S	S/S/S	
	5	7		1		3
2	5	6		1		4
2	1	6	1		1	1
3	4	5	1	1		1
8	2	3		1	1	1
					Total	10 cells

1) H = Hordeum chromosomes
2) S = Secale chromosomes

Levan (1942) observed differences in meiotic behaviour of the chromosomes in three haploid rye plants, similar to those described above for the two H. jubatum x S. cereale hybrids. This is of considerable interest, since the haploid rye chromosomes in the hybrid are apparently behaving in similar fashion as in haploid rye plants. The behaviour of the rye chromosome is thus independent of the complement of Hordeum chromosomes in the hybrid.

Anaphase I

The anaphase I stage showed very irregular behaviour due to the many univalents in each cell. The regularity of movement of the Hordeum chromosomes to the poles depends on the number of bivalents in metaphase I. If the number of bivalents is lower than seven, Hordeum univalents will contribute to the irregularity of the cell division.

The irregularities consist of lagging of univalents followed by late splitting into chromatids at the equatorial plate, while the bivalent partners have almost reached the poles (Fig. 16). The rye chromosomes move randomly to the poles or lag partly and split into their chromatids at the metaphase plate. Fig. 16 shows a cell in which one pole has two rye chromosomes, the other only one. Each pole has four Hordeum chromosomes. Laggards between the two poles consist of eleven chromosomes: five rye chromosomes of which three have already split and six Hordeum chromosomes (one lies on top of one of the rye chromosomes).

Often, however, each pole shows seven Hordeum chromosomes with a number of rye chromosomes varying from zero to seven. The distribution of the rye chromosomes towards the poles depends, of course, on the position of the univalents in the metaphase I cell. If they are all located at one side of the metaphase plate (Fig. 17), then a telophase as shown in Fig. 18 may arise. One pole will contain many chromosomes and one with only a few, resulting in a large cell with a large nucleus and a small cell with a small nucleus at the interphase. Usually, however, the two cells of a diad are more or less of the same size. Extreme cases, as mentioned and illustrated by Brink et al. (1944a, Fig. 3, J and N), in which one pole receives 20 or 21 chromosomes, have not been encountered in this material, although their occurrence is possible.

Bridge formation was observed sometimes at anaphase I. This appeared to be stronger in hybrid 134-57. In this hybrid the cells at A. I stage were difficult to analyze.

Interphase

At interphase the diad cells often contained micronuclei, caused by failure of lagging chromosomes to reach the poles (Fig. 19, 22). The number of micronuclei was found to vary from none to several per cell. Fig. 22 shows a case in which the two cells of the diad have formed several micronuclei. Microcyte formation between the diad cells occurred very rarely. This is in contrast with the frequent occurrence of microcytes in hybrids as those between Triticum timopheevi and other tetraploid wheat species as T. durum, T. dicoccoides, etc. (see Part II).

Second division

From interphase onward in the anthers of many florets a process of generation started, which led to the death of the entire contents. It was noticed that in many heads the P.M.C's died before reaching the tetrad stage. The beginning of this process can be seen in Fig. 21. The cells were apparently dying as judged by the rather thick appearance and irregular outline of the cell wall. In many anthers, however, the cells were able to reach metaphase II stage (Fig. 23 - 28). In these cells the rye and barley chromosomes were still distinguishable. Most chromosomes are separated into their chromatids as can be seen in these pictures. Chromatid pairs of the same size lay close together. Some of the chromosomes, however, can be seen as single chromosomes. These were chromosomes which probably were univalents in metaphase I and divided into their chromatids when lagging in the anaphase. They were not able to divide further at metaphase II, as is shown by their single appearance.

Metaphase II

Metaphase II cells are usually difficult to study. In this material it appeared to be rather easy. This is due to two causes:
(1) The chromosomes are very short and spiralized.
(2) These same cells (in the degenerating stage) fail to form a spindle and the chromosomes are scattered throughout the cytoplasm. The chromatids of the split chromosomes fail to go to the poles and stay where they are (Fig. 24, 25, 26, 27, 28). An interesting feature of these cells - that fail to form a spindle and to reach stages beyond

the metaphase II - is that they almost always show a nucleolus (Fig. 24 - 27). The nucleolus has never been seen at metaphase I and anaphase I in non-degenerating cells. Moreover, such cells were always able to go through these stages with normal development of a spindle.

This evidence may indicate a nucleolus - spindle relation. Spindle formation will occur after disappearance of the nucleolus (Gates, 1942). In the metaphase stage the spindle is formed and the chromosomes are lined up in the equatorial plate. If spindle formation fails to occur the nucleolus remains visible; the chromosomes remain in the prometaphase stage, lining up in the equatorial plate and subsequent divisions fail to occur. The relationship between nucleolus and spindle formation has been reported previously by Walker (1957) who did experiments on living pollen cultures of Tradescantia treated with colchicine. The observations on the metaphase II cells of the H. jubatum x S. cereale hybrids seem in accord with his theory. There are, however, several cases reported where the nucleolus persists during metaphase and anaphase of mitosis (see Brown and Emery, 1957). Spindle formation seems here to be independent of the nucleolus. It is therefore perhaps better to assume that the stage observed in degenerating P.M.C's of the hybrids is actually prometaphase and that development ceased at this stage.

Many cells at metaphase II stage did not appear, as is usually the case, in the diad condition. Several of them were single and rounded off (Fig. 25, 27, 28, 29, 30, etc.). Originally it was thought that these cells were pollen grains at first mitosis but this interpretation was abandoned for three reasons. In the first place it appeared

that slightly younger anthers had not yet completed meiosis and had not gone through the tetrad stage; secondly, diads, with prometaphase characteristics as those of single cells, occurred together with them in a single anther; thirdly, in the prometaphase of these single rounded cells, single chromosomes appeared in the same cell with chromosomes that had separated into daughter chromatids. If these cells were in pollen mitosis, these single chromosomes - often large rye chromosomes - would be absent. Unable to divide at metaphase II they would have been left behind on the metaphase plate and thus been excluded from the telophase II nuclei. Pollen mitosis would thus have shown a regular double structure for all chromosomes. Since single chromosomes appeared regularly in the cells concerned, it may be concluded that these chromosomes had already undergone a chromatid division (M. I), but were not yet eliminated from the nucleus by the second meiotic division.

The single cells concerned apparently originated from diad cells which rounded off and separated. Several diads, as in Fig. 21 and 23, were noticed with the two cells not completely separated.

As stated earlier, spindle failure prevents anaphase II grouping at the poles. However, a kind of random regrouping and nucleus formation often occurred. Chromosomes lying closely together formed a nucleus without including scattered single chromosomes. This process is clearly demonstrated in Fig. 29 and 30. Fig. 29 shows a cell with three nuclei, and six single chromosomes. These chromosomes were probably just too remote to be included in one of the three nuclei. Fig. 30 shows an extreme case: here several of the smaller nuclei appear to contain not more than one or two chromosomes.

Later the cells die off rapidly, although many degenerating cells cannot form these random "nuclei" and the chromosomes remain visible until the whole cell contents have disappeared (Fig. 26, 31, 32, 38). In the degenerating process the cells lose their healthy, turgid appearance; the cell walls become loose and exhibit folds (Fig. 26, 31, 32, 33). The amount of cytoplasm decreases, and in somewhat older material only empty cell walls are found.

Only in very few anthers were cells in anaphase II and telophase II encountered (Fig. 34, 36, 37). Irregularities such as lagging chromosomes and bridges were often observed in these cells. Figure 34 represents an anaphase II cell in which the chromosomes were countable and distinguishable. Although many anthers were studied, only one tetrad was encountered (Fig. 35).

From the observations described above it is clear that the pollen mother cells of these hybrids are intrinsically able to go through the entire second meiotic division and to form tetrads. Krishnaswamy (1939) reported degeneration of several P.M.C's before completion of the second division in a haploid of Triticum vulgare. In the hybrid discussed here the degeneration was apparently not dependent on the physiologic state of dividing cells, but on the physiological state of the anthers. If any pollen mother cells in an anther showed signs of collapse then all cells in the anther showed the same degeneration. If any cells are able to go through metaphase II, anaphase II, etc., to the tetrad stage, all cells of that particular anther were apparently able to do so. The author got the impression that this can even be extended to heads; in most heads the P.M.C. contents

in all anthers degenerate at an early stage before completion of the meiosis; in a few heads somewhat later stages of development may be reached by the P.M.C's. This would tend to confirm the belief that the early degeneration process of the cells, in meiotic division, is of a physiological, nutritional nature, quite likely due to failure of proper functioning of the tapetum cells. Degenerate and collapsed tapetum cells were indeed observed in the anthers concerned. Crowder (1953) observed in his Festuca - Lolium hybrids the same phenomena: cells which do not complete their meiotic cycle and degenerate, probably due to break down of tapetum cells. He also observed inferior tapetum cells.

Giant cells

One other curious phenomenon may be mentioned: giant cells two to six times the diameter of normal cells with more than the normal complement of chromosomes. Giant cells with more chromosomes than the ordinary number due to cell fusion (syncytes), have been previously described by several authors (Gaines and Aase, 1926; Levan, 1941; Müntzing and Prakken, 1941, Krijthe, 1942, Crowder, 1953). Müntzing and Prakken (1941) working with rye assumed that cell fusion had taken place between leptotene and metaphase I since at metaphase I only bivalents were observed.

In the present hybrid material, giant cells were only observed in the three anthers of one floret. In these three anthers 14 large cells were seen (Fig. 38 - 45). The available material was therefore very limited, and how they originated could not be established with certainty. Figures 38 - 40 show large cells with extra chromosomes

at metaphase I stage. Two interesting things can be seen: (1) All of these syncytes show a very granular cytoplasm, which is also observed in degenerate cells of normal size (Fig. 20, 21, 23, 26, 28, 31, 32, 33, 35, 36, 37). (2) The cell in Figure 38, although probably in metaphase I, does not show any bivalent formation, the chromosomes are scattered around, and 3 nucleoli are visible. Although the actual stage of the cells in Fig. 41, 42 and 43 are difficult to establish they are believed to be at anaphase I stage. Nucleoli are visible here. The cells in Fig. 39 - 40 show definite bivalent formation.

Several of these large syncytes show a relatively small protrusion (Fig. 40 - 43), with or without chromosomes. The origin of these protrusions is a matter of speculation since not enough material was available to investigate its actual nature. There are two possibilities:

(a) The protrusion is the remainder of a cell fused into the larger one. It can be seen that there is often a concentration of chromosomes near or in the protrusion. Chromosomes belonging to the fused cell may be moving into the large one. Fig. 44 and 45 show cutting off of a certain amount of cytoplasm (with or without chromosomes). This may have occurred before the entire cell contents had been moved into the large cell.

(b) The protrusion is a result of abnormal physiological condition of the large cells (this phenomenon is only observed on the large cells) and is actually an outgrowth of the syncyte. The chromosomes may or may not move into the protrusion (Fig. 40, 41, 42, 43). A kind of "budding off" may be followed by cell wall formation between

the cell and protrusion. This may lead to situations shown in Fig. 44 and 45.

Although at first cell fusion seems more likely than protrusion formation , a few points may favour the latter condition. In each observed case the chromosomes in or near the protrusion are in the same stage of division as those in the large cell. In case of cell fusion, this would not always be necessary since some fluctuation in the stage of division was quite common in these anthers. Furthermore, no evidence of a cell wall having existed around the "bud" can be seen. More material with additional evidence is, however, necessary to throw a clear light on this phenomenon.

Whether Fig. 46 to 49 represent the same phenomenon as just discussed is not certain. They may represent a sequence of cell types, in which three chromosome groups have been formed. Figure 46 indicates how such a cell may arise: in a cell with a higher number of chromosomes than normal, a group of univalents are located outside the spindle action: they form a group of chromosomes besides the two polar groups. The form of the spindle does not indicate a three-polar spindle. Cell walls are formed between each group to produce the three-cell associations.

As already stated, it is clear that these unusual phenomena result from the abnormal physiologic condition in the anther tissues. Haploids, triploids, etc., often show similar phenomena (Gaines and Aase, 1926; Levan, 1941, etc.). The triploidy of this hybrid, together with the fact that chromosome sets of two different species are brought together is probably the basic cause of the upset of the physiologic mechanism of the anthers.

Plate II

Fig. 2. Spikes of H. jubatum, the hybrid H. jubatum x S. cereale and S. cereale.

Fig. 3. Spike of the hybrid, H. jubatum x S. cereale, showing the open, sterile florets.

Fig. 4. Spikelets of H. jubatum (A), S. cereale (D) and their hybrid (B and C).

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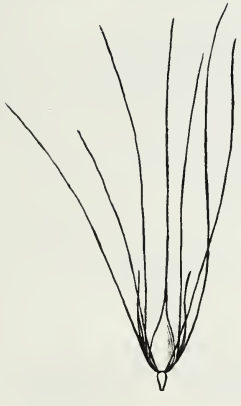

A

H. jubatum

B C

Hybrid

D

S. cereale

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Plate III

Fig. 5. Metaphase I cell of *H. jubatum* (2n = 28) with 14 bivalents.

Fig. 6. Early metaphase I cell of *S. cereale* (2n = 14) with seven bivalents.

Fig. 7 - 15. Metaphase I cells of the hybrid, *H. jubatum* x *S. cereale*. The *S. cereale* parent is the var. Antelope except in Fig. 14 in which var. Sangaste was the rye parent. Arrows indicate the smaller *Hordeum* univalents.

Fig. 7. Six bivalents and nine univalents, the two small univalents being *Hordeum* (arrows) and seven large ones the rye chromosomes.

Fig. 8 - 9. Seven bivalents and seven (rye) univalents. Note close (autosyndetic) bivalents of *Hordeum* chromosomes.

Fig. 10. Six bivalents and nine univalents. Two rye chromosomes show an e - e association. Arrows to the two *Hordeum* univalents.

Fig. 11. Five bivalents and 11 univalents. One rye and one *Hordeum* chromosome show an e - e association and two *Hordeum* chromosomes a s - s association.

Fig. 12. Eight bivalents (of which one involves two rye chromosomes) and 5 *Secale* univalents.

Fig. 13. Five bivalents (of which one is a "rye bivalent") and 11 univalents, with two rye univalents in s - s association.

Fig. 14. Few cells showing tangled chromosomes making interpretation difficult (F_1 *H. jubatum* x *S. cereale* var. Sangaste).

Fig. 15. Five bivalents, one (*Hordeum*) trivalent and eight univalents of which seven are rye chromosomes (arrow to Hordeum univalent).

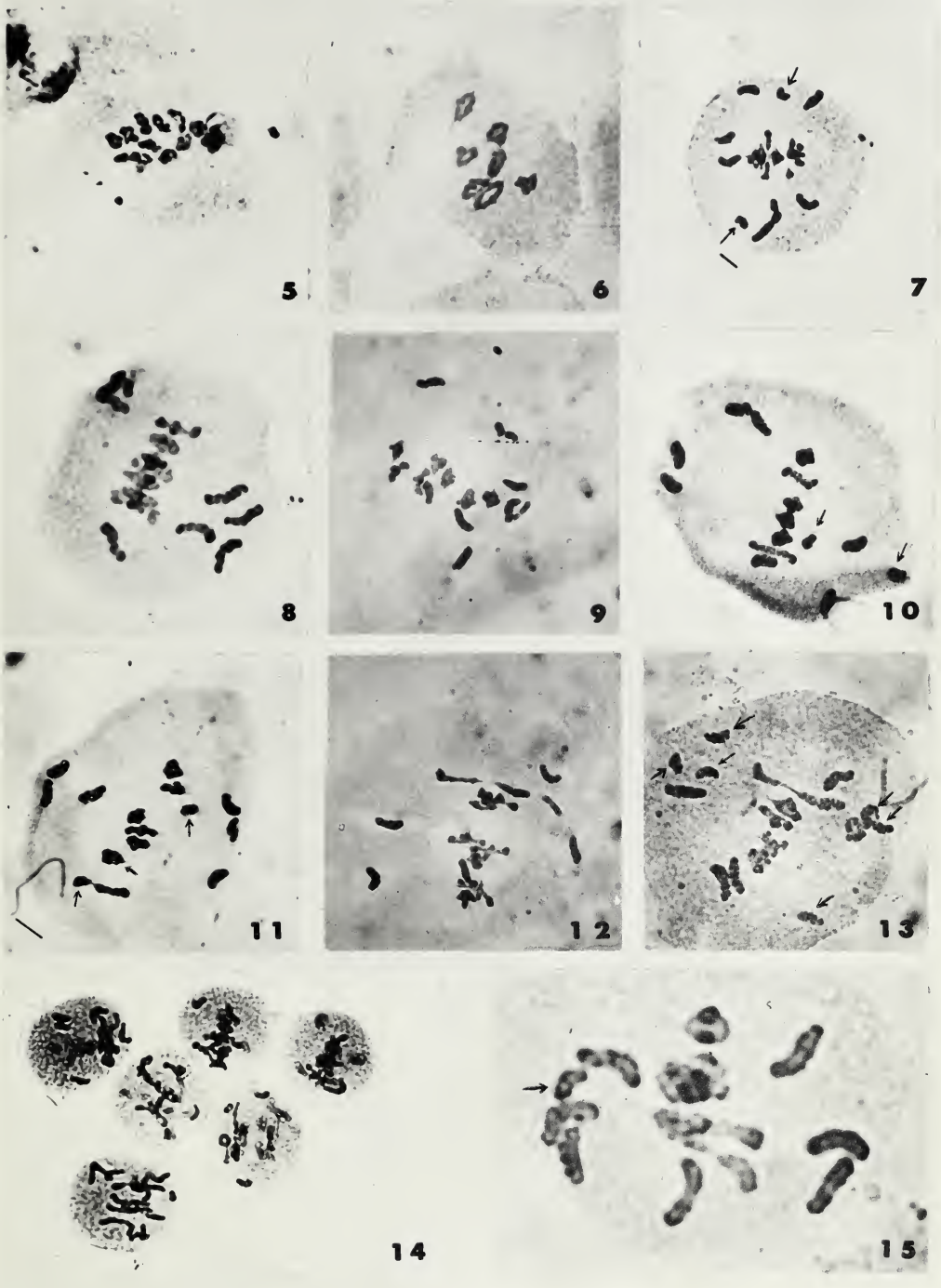
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Plate IV. All figures are of cells of the F_1 *H. jubatum* x *S. cereale* var. Antelope except in Fig. 23 in which the var. Sangaste is the rye parent.

Fig. 16. Anaphase I cell with 11 lagging univalents of which five are rye (three already split) and six are *Hordeum* chromosomes (one lies on top of a rye chromosome).

Fig. 17. Metaphase I cell with most of its univalents at one side of the metaphase plate.

Fig. 18. Fig. 17 will probably lead to a telophase I cell as pictured here, with one large and one small nucleus.

Fig. 19. Interphase: diad with one micronucleus.

Fig. 20. Interphase: one diad.

Fig. 21. Interphase diad with rather thick cell walls and becoming separated, although still attached.

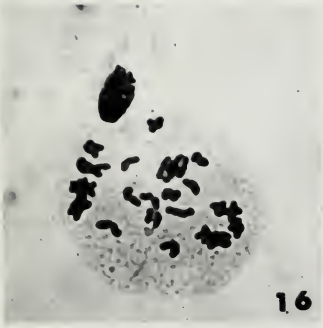
Fig. 22. Prophase II cell with several micronuclei.

Fig. 23. Metaphase II: cells of diad rounded but still attached.

Fig. 24. Metaphase II diad; most chromosomes split into chromatids, giving the double structures.

Fig. 25. Similar to Fig. 24; note size differences of *Hordeum* and *Secale* chromosomes; some single chromosomes (arrows) and nucleoli.

Fig. 26. Degenerating M II cells, double chromosome structures still noticeable. Note granular cytoplasm.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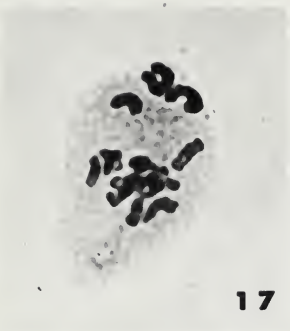
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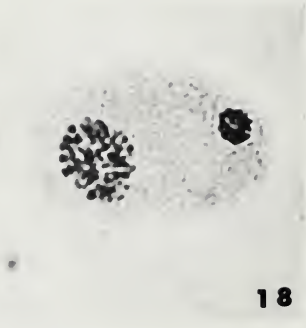
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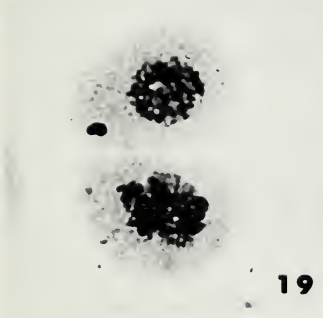
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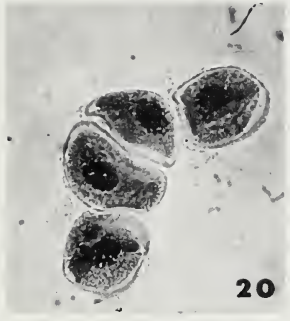
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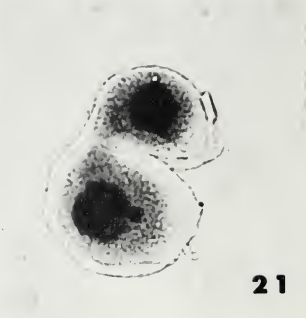
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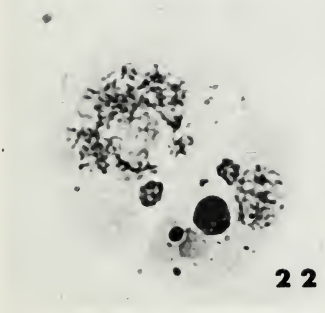
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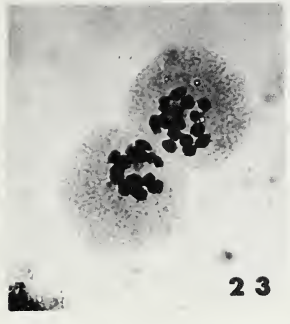
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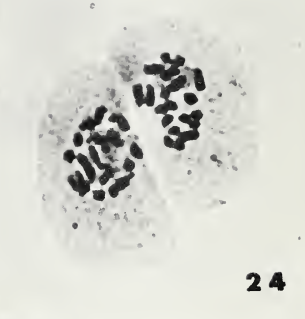
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Plate V

Fig. 27, 29, 30, 34 show cells of F_1, H. jubatum x S. cereale var. Antelope.

Fig. 28, 31, 32, 33, 35, 36, 37 are of cells of F_1, H. jubatum x S. cereale var. Sangaste.

Fig. 27. Metaphase II in a rounded cell simulating pollen mitosis (note single rye chromosomes, arrows).

Fig. 28. Similar cell is in Fig. 27 in later stage and disintegrating.

Fig. 29. Chromosomes grouped into three nuclei with six separate chromosomes.

Fig. 30. Extreme case of "nuclei-formation" by small groups of chromosomes.

Fig. 31. Metaphase II cell degenerating: chromosomes still visible.

Fig. 32. Similar to Fig. 31, a little later.

Fig. 33. Degenerating diad (metaphase II); in left cell chromosomes still partly visible.

Fig. 34. The only good anaphase II cell encountered in this material. Seven small Hordeum chromosomes are going to upper pole, six of the same to the lower pole. One lies among the six large rye chromosomes between these two groups.

Fig. 35. The only tetrad found, with several micronuclei.

Fig. 36. Anaphase II and telophase II cells, the latter with laggards.

Fig. 37. Telophase II cells forming cell walls; several laggards are present.


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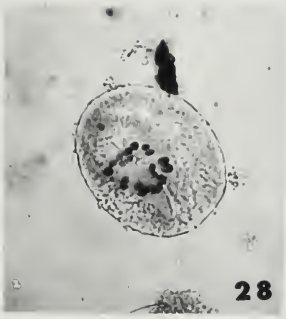
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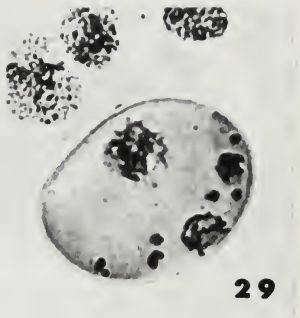
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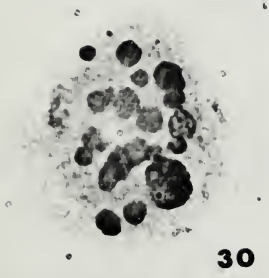
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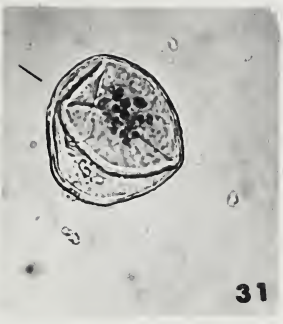
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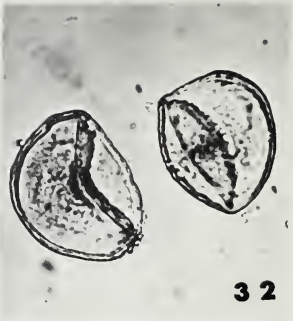
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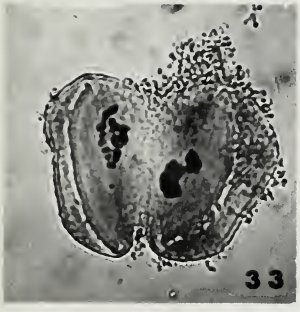
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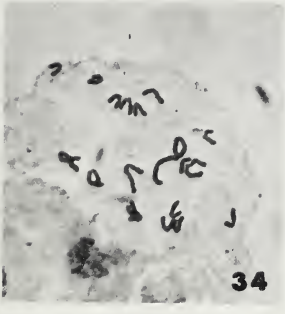
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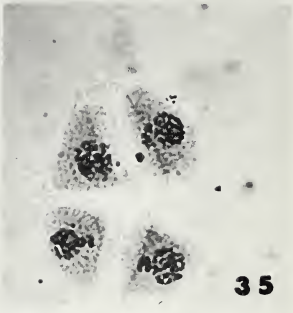
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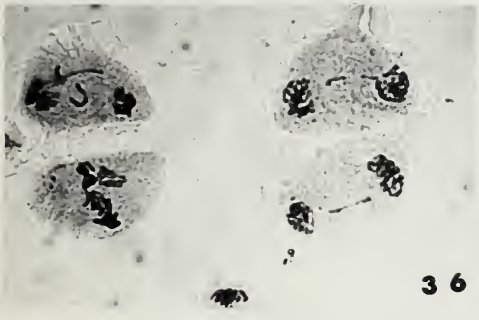
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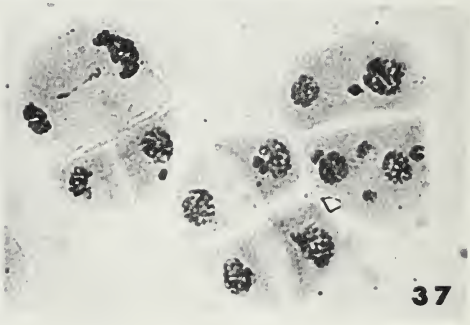
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Plate VI. Giant cells with supernumerary chromosomes. Fig. 38 - 45 were observed in one floret of the hybrid, H. jubatum x S. cereale var. Antelope. Fig. 46 - 49 are from the F_1, H. jubatum x S. cereale var. Sangaste.

Fig. 38. Giant cell, probably in metaphase I stage.

Fig. 39. Giant cell, at metaphase I with bivalents.

Fig. 40. Same as Fig. 39, with protrusion including a group of chromosomes.

Fig. 41. Protrusion without chromosomes. Stage of cell difficult to determine. Note nucleolus.

Fig. 42. Giant cell with three groups of chromosomes; a fourth group in tube. Note granular cytoplasm, also in Fig. 38 - 41.

Fig. 43. Same as 42. Dark round body is nucleolus.

Fig. 44. Giant cell in telophase I stage, with small cell (including 4 chromosomes) which has been attached to large cell (arrows).

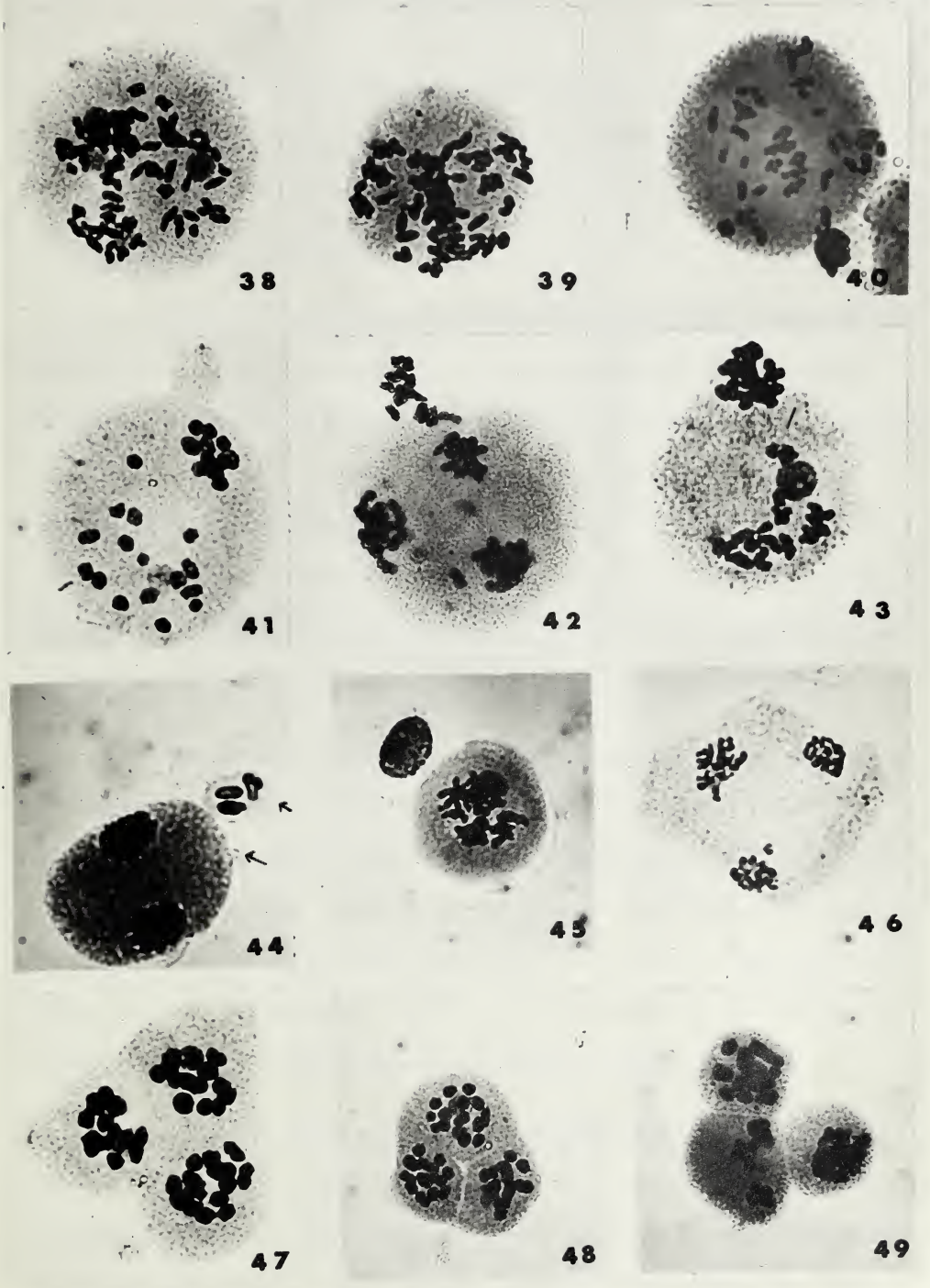
Fig. 45. Giant cell with small cell attached, containing only cytoplasm.

Fig. 46. Telophase I cell with extra chromosomes. One group of chromosomes (univalents) fell outside spindle.

Fig. 47. Cell with three groups of chromosomes probably forming separate cells.

Fig. 48. Similar to Fig. 47, but now with cell wall formation between chromosome groups.

Fig. 49. Probably similar to Fig. 48, one stage later with cells partly separated.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D. DISCUSSION

Certain of the results demand further consideration:

(a) The embryonic development of the hybrid zygote.

(b) The possible consequences of the observed autosyndesis of the *H. jubatum* chromosomes.

After fertilization the embryo develops slowly but normally. According to Brink and Cooper (1944) the development of the hybrid embryo is only slightly behind that of the selfed embryo of *H. jubatum*. They found, however, evidence that the physiological mechanism of nutrition of the hybrid seed is defective and that the hybrid embryo breaks down after 13 days as a result of starvation alone. Replacement of this embryo in an artificial nutrient medium would give the embryo a chance to continue its development and eventually to germinate.

The present author's observations do not entirely agree with the descriptions given by Brink and Cooper. The hybrid embryo at 11 to 12 days of age appeared to be definitely much smaller than the selfed controls. The latter showed development of a scutellum, a feature that the hybrid embryo of the same age completely lacked. This would indicate that in this material the rate of development of the hybrid is significantly lower than that of the selfed embryo.

Although Brink and Cooper concluded that the development of the hybrid embryo was only a little behind the normal of the same age, culturing of hybrid embryos showed only a very little germination and survival percentage. These authors suspected, therefore, that the failure of differentiation in most of the hybrid embryos was due to an

intrinsic defect of the embryo of certain plant hormones essential to the very young embryo for continuation of its development rather than an underdevelopment of the embryo (van Overbeek, 1942; van Overbeek et al., 1942). Addition of growth substances to the nutrient medium could possibly provide the embryo's requirement.

It is, however, noteworthy that selfed embryos, 8 to 12 days old, serving as controls in the author's experiments, showed almost 100% germination. This is in striking contrast to the very low germination rate of the hybrid embryos. Addition of coconut milk as suggested by various workers (Brink et al., 1944a; van Overbeek, et al., 1942) as sources of plant hormones did not produce any improvement in the differentiation, and thus in the germination, of the hybrid embryos. It is therefore believed that the low germination rate of the hybrid embryos is related also to other physiological and probably genetic factors. The main factors involved are assumed to be:
(1) underdevelopment and undernourishment of the embryo
(2) physiologically lower efficiency (in metabolism) due to genetic imbalance of the hybrid embryo. Embryos were therefore weak and more susceptible to the shock of transplantation.

The second point that needs some discussion is the observed autosyndesis of the H. jubatum chromosomes and some of the related problems. That autosyndesis of the H. jubatum chromosomes occurred could be established because of the striking size differences of the rye chromosomes. This important feature of hybrids with Secale cereale as one of the parents was emphasized by Stebbins and Fung (1953). Although some other workers have used this feature in their hybridization

experiments to establish the amount of autosyndesis (Gaul, 1953, Melnyk, unpublished), the merits of S. cereale are not sufficiently realized. Besides the large chromosomes, it may be mentioned that S. cereale crosses relatively easily with many genera of the tribe Hordeae. Gaul (1953) using rye as "analyzer" (Kihara and Nishiyama, 1930) assumed that the differentiation of the chromosomes of Secale proceeded more rapidly than the mechanism hindering crossability with other genera. Little or no genome similarities can be expected therefore in Secale hybrids, and if pairing occurs it is only due to autosyndesis. This view of Gaul can be disputed since it seems reasonable to assume that crossability is strongly correlated with the phylogenetic differentiation of the chromosomes. Furthermore, occasional bivalent formation between Hordeum and Secale chromosomes in the hybrid H. jubatum x S. cereale (134-57) makes his statement doubtful. The author believes that the difference in chromosome size is more important than genome differentiation as a cause of intergeneric pairing failure in the intergeneric hybrids involving rye. Whatever the explanation may be, rye has some favourable features when used in intergeneric crosses.

In a recent paper Stebbins (1956) summarizes all the intergeneric crosses within the tribe Hordeae so far done (and known). The outstanding crossability of rye is striking: Secale has been successfully crossed with species of the genera Triticum, Aegilops, Haynaldia, Agropyron, Hordeum and (by the present writer) Elymus. The author is convinced that this list can be extended, although embryo culture may be necessary for obtaining hybrids.

The question whether the Secale genome has an effect on the autosyndetic pairing of the other set of chromosomes in these hybrids is of considerable significance in the field of genome analysis. On the basis of studies on F_1 hybrids between S. cereale and Agropyron species Gaul (1953) and Stebbins and Fung (1953) concluded that the Agropyron chromosomes paired autosyndetically and that this pairing was unaffected by the presence of rye chromosomes. Evidence that the rye chromosomes behave similarly to true haploids is obtained in the hybrids H. jubatum x S. cereale (see pages 18 and 19).

That such a genic interference with bivalent formation (asynapsis, desynapsis) exists between genomes of certain species is generally known. Gaul (1954) has recently reviewed and discussed the extensive literature on this problem. Evidence regarding effects on autosyndetic pairing within H. jubatum chromosomes is given in the H. jubatum x H. vulgare hybrid made by Mr. E. R. Kerber (personal communication) who observed mostly only two to three loose bivalents at metaphase I. It seems now quite likely, in view of the observed autosyndesis in his H. jubatum x S. cereale hybrids, that this low bivalent formation is best explained as a genic interference of the H. vulgare chromosomes with the pairing or chiasma formation of the H. jubatum chromosomes. To verify this some hybrids between the strain of H. jubatum used by Kerber to obtain his hybrid with H. vulgare and S. cereale var. Prolific have been produced by the writer. Unfortunately, due to very slow growth, data fromthese hybrids are not yet available.

Another example of genic interference with bivalent formation has recently been found by the present author in the hybrid H. jubatum x

H. bulbosum. H. bulbosum (2n = 28) is assumed to be a true autotetraploid (von Berg, 1948; Stebbins, 1957) and one would expect to observe 11 - 14 bivalents in a hybrid between H. jubatum and H. bulbosum. However, in the hybrid of these two species obtained by the author, the bivalent number varied from zero to seven bivalents with a mode at four bivalents.[1] Apparently, the genic interference is so strong that the number of bivalents observed in the hybrid was considerably less than expected.

Such a genic interference on bivalent formation - whatever the actual cause - may lead to wrong conclusions in genome analysis. The chromosome pairing in Kerber's hybrid between H. jubatum and H. vulgare may be used to illustrate this. On the basis of observations of meiosis one would be inclined to assume true allopolyploidy for the species H. jubatum. The present study on the H. jubatum x S. cereale hybrid indicates that this is probably not the case. One may assume that such situations will occur more frequently and recognition of possible interference of (a) asynaptic gene(s) in genome analysis seems to the present author of great importance. Critical conclusions on the origin of certain species will therefore only be obtained if based on a series of crosses rather than on a single cross.

The observed autosyndeses in the author's H. jubatum x S. cereale hybrids appear to be the result of close relationship of the two genomes in H. jubatum. The pairing was fairly close although not perfect: the average was about 6 bivalents per cell of which 2.40 bivalents were open and 3.53 were closed (Table III). The fact that in the metaphase I of the meiosis of the species H. jubatum no multivalents

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[1] See Fig. 69 and 70.

were observed suggests preferential pairing of the chromosomes within each genome in the tetraploid condition.

In the artificially induced hybrid between Agropyron trachycaulum (2n = 28) and H. jubatum (2n = 28) Boyle and Holmgren (1955) observed an average of 6.3 bivalents per cell of which 89% were open bivalents. On the basis of complete absence of quadrivalents in either H. jubatum or A. trachycaulum, and the low number of quadrivalents in the induced octoploid (2n = 56) of that hybrid (Ashman and Boyle, 1955), allosyndesis between chromosomes of an Agropyron genome and a Hordeum genome were assumed. They proposed a genome formula for the tetraploid hybrid of AABC, for A. trachycaulum AABB and H. jubatum AACC.

It should be emphasized here that no size differences of chromosomes were noted in their hybrid, thus making it very difficult to interpret the data. Additional evidence was therefore needed to support their hypothesis. Boyle and Holmgren took this evidence mainly from the lack of quadrivalent formation at meiosis in the respective parental species. This criterion, used in deciding between auto- or allosyndesis in certain species, is, however, a rather dangerous one without the support of additional evidence. Segmental allotetraploids (originating from closely related species with much genome homology) may show only bivalent formation at meiosis, although in the haploid condition this segmental allotetraploid would probably show autosyndesis. Stebbins (1950) in describing and reviewing the cases of supposed segmental allotetraploids, explains that selection pressure on the "raw" segmental allotetraploid in favour of increased fertility

may increase quadrivalent or bivalent formation. In the latter case, although two sets of genomes with much homology exist together, preferential pairing of the chromosomes will result in complete bivalent formation. If the four genomes were very similar or identical (as in an autotetraploid) one would expect quadrivalents in the tetraploid species, as is observed in H. bulbosum (v. Berg, 1948). Since quadrivalents were not observed either in H. jubatum or A. trachycaulum Boyle and Holmgren (1956) decided that the bivalent formation in the hybrid must have been allosyndetic, overlooking the possibility of segmental allopolyploidy for one of the species.

In the author's opinion the criterion of quadrivalent formation in an autotetraploid may not always be valid. Nordenskiöld (1945) reported in the autopolyploid Phleum pratense (2n = 42) no multivalent formation, only bivalents being observed. In trying to obtain Phleum pratense artificially, she treated P. nodosum (2n = 14) twice with colchicine (Nordenskiöld, 1949). The artificial hexaploid thus obtained showed bivalents, and a few univalents. A quadrivalent was observed in only a few cells. In cases, as mentioned above, it is expected that chromosomes of such plants in the haploid condition, with or without additional chromosomes of another species (as in hybrids), will exhibit autosyndesis.

These points were probably overlooked by Boyle and his coworkers (Boyle and Holmgren, 1955; Ashman and Boyle, 1955) when they concluded allosyndesis existed between chromosomes of H. jubatum and A. trachycaulum.

As mentioned before, bivalent formation among the H. jubatum chromosomes in the hybrid H. jubatum x S. cereale was fairly close. The proportion of rod bivalents (40%) was considerably lower than in the A. trachycaulum and H. jubatum hybrid (89%). This difference may be due to genic effects on chiasma formation in the latter hybrid.

In considering the origin of H. jubatum two possibilities exist: (1) H. jubatum is an autotetraploid or (2) H. jubatum is a segmental allotetraploid, thus originating by natural hybridization and spontaneous chromosome doubling from two closely related Hordeum species. On present knowledge, however, it seems that autotetraploidization of a diploid H. jubatum is unlikely. The autosyndesis observed in the hybrid is probably not strong enough for true autotetraploidy (in which seven bivalents would be expected to occur regularly), since only 24% of the metaphase I cells of the hybrid showed seven bivalents. Assumption of some structural differences in the two genomes of H. jubatum seems logical. Furthermore, it seems that the probabilities of autotetraploids arising in an inbreeding species such as H. jubatum are very small. Stebbins (1956) pointed out that true autotetraploids have so far been found only in perennial, outcrossing species. As examples he mentioned Dactylis glomerata, Hordeum bulbosum, Agropyron cristatum, etc. In inbreeding species no evidence of true autotetraploidy has been found. Stebbins points to this rather paradoxical situation when he explains that the main factor in establishing a new natural autopolyploid is the success of its survival after its formation, not the ease with which it can be formed. This success of survival seems to be the greatest in plants of hybrid origin, or at least those with a great deal of heterozygosity.

On the basis of what has been said above the most logical conclusion is that H. jubatum is a segmental allotetraploid that apparently originated from two diploid species having very similar genomes. Some structural differences, however, must have existed between the chromosomes of the two genomes. Furthermore, Stebbins (personal communication) mentions the existence of reasonably fertile hybrids which he supposes to have originated from hybridization between H. jubatum and H. brachyantherum. He suspects that either H. californicum (2n = 14) or an extinct diploid species - related to H. compressum or H. stenostachys and supposed to be the ancestors of H. brachyantherum (Covas, 1951) - was one of the diploid ancestors of H. jubatum. Critical evidence of the origin of H. jubatum can thus only be obtained if H. jubatum is hybridized with these various species and, as Stebbins suggests, also with synthetic autotetraploids derived from them.

E. SUMMARY

1. Four intergeneric hybrids of the cross, H. jubatum (2n = 28) x S. cereale (2n = 14), were grown, early development being made possible by embryo culturing in an artificial nutrient medium.

2. Two of these hybrids, with rye parents var. Antelope (33-57) and var. Sangaste (134-57), respectively, were cytologically investigated.

3. Both hybrids had the somatic number of 21 chromosomes.

4. The rye chromosomes were larger and therefore distinguishable from the smaller H. jubatum chromosomes.

5. In both hybrid plants fairly strong autosyndesis of the H. jubatum chromosomes (average 5.9 bivalents and 9.2 univalents) was observed.

6. In the hybrid having var. Antelope as the rye parent, bivalents of rye chromosomes were rarely observed (1 in 31 cells). However, in the hybrid, with var. Sangaste as the rye parent, rye chromosomes regularly formed some bivalents and multivalents, both autosyndetically and in conjunction with H. jubatum chromosomes.

7. Pollen mother cells of the hybrids often degenerated before completing meiosis. Tetrads were observed to form only very rarely. This process of degeneration of the pollen mother cells was attributed to disturbance of the nutritional function of the tapetal cells.

8. The observed autosyndesis of H. jubatum chromosomes in the hybrids indicates that H. jubatum possesses two very closely related genomes and that this species may be considered as a segmental allotetraploid.

PART II

CYTOLOGICAL STUDIES

ON THE G-GENOME OF *TRITICUM TIMOPHEEVI* ZHUK.

PART II

CYTOLOGICAL STUDIES ON THE G-GENOME OF TRITICUM TIMOPHEEVI ZHUK.

A. INTRODUCTION

The question of whether the methods of genome analysis lead to reasonably accurate conclusions regarding the origin and relationships of various members of a plant taxon has arisen in recent years. The genome concept, and the use of genome symbols, were introduced by Kihara (1924, 1930) in his early work on the wheats. He also laid the basis for the method of genome analysis. According to this concept, the degree of pairing and bivalent formation at metaphase I of the interspecific or intergeneric hybrid indicates the degree of chromosome homology. In particular, it is this bivalent formation in the hybrids which gives an index of genome homology and thus of species relationships, although caution is necessary in interpreting observations of chromosome behaviour in hybrids.

Sax (1935), discussing the factors involved in genome analysis, writes:

> "Changes in chromosome structure are known to be an important factor in changing the homology of chromosomes of different individuals or species. Segmental interchanges, inversions and loss or duplication of chromosome segments may change the chromosome structure of different species to such an extent that no two chromosomes in the species-hybrids are completely homologous. Slight changes would not inhibit regular pairing, but the hybrid might be partially or completely sterile owing to segmental deficiency following random chromosome distribution at meiosis."

This type of sterility with a normal chromosome behaviour at meiosis of the hybrid is later called by Stebbins (1945, 1950) cryptic structural hybridity.

It is well-known, however, that failure of chromosome pairing (asynapsis) can be caused by genetic (Part I, page 32) and environmental factors as well as by lack of sufficient homology. Moreover, if pairing and bivalent formation is incomplete one has to be very careful in postulating relationship. Other factors such as hybrid sterility and chromosome morphology should then be considered. That association formation in the metaphase I of a hybrid always means chromosome homology is not necessarily true. Levan (1942) observed non-homologous chromosome pairing in haploid rye. Sears (1947) points out, however, that usually very few such associations persist until metaphase I, where pairing in the hybrid is commonly studied.

In genome analysis, conclusions are therefore often of an arbitrary nature, whereas the use of genome symbols tends to oversimplify the relationships. With our relatively rough methods of genome analysis these designations are usually sufficient. Sometimes, however, a deeper study makes it necessary to reconsider or even to alter the original conclusions. In particular the differentiation between genic or environmental effects versus the effect of lack of homology as causes of pairing failure may be possible only after studying a series of crosses, rather than a single cross between the species and genera concerned.

Certain findings relating to genome analysis of the relatives of the tetraploid species of wheat may indicate that factors other than genome homology are operating to cause lack of pairing in hybrids. These tetraploid (2n = 28) species are usually divided into two groups. The largest is the Emmer group, which includes T. durum, T. dicoccoides,

T. polonicum, T. persicum, etc. These species, when intercrossed, produce hybrids that show regular chromosome behaviour at meiosis and high fertility. The second group (Timopheevi group) consists of two species, T. timopheevi and T. armeniacum. If either of these species is crossed with species of the Emmer group, hybrids show a very irregular meiosis and high sterility. It is mainly on the basis of these characteristics that these two species were placed in a separate group (Lilienfeld and Kihara, 1934; Svetozarova, 1939).

The species T. timopheevi was identified by Zhokowski as having 28 chromosomes, and was at first grouped in the Emmer series of wheat. Lilienfeld and Kihara (1934) crossed T. timopheevi with the diploid T. monococcum (2n = 14, AA) and the tetraploid Emmer wheat T. persicum (2n = 28, AABB). At meiosis of the first hybrid (2n = 21) they observed seven bivalents and seven univalents, indicating one common genome in T. monococcum and T. timopheevi. In the meiosis of the second hybrid, a range of from 9 to 14 bivalents (mostly 12) was observed, indicating again one common genome (the A genome) and one only partially homologous genome. The hybrids showed very low fertility (4.8%). On the basis of chromosome pairing at meiosis and the observed sterility of the hybrids, they assumed that the second genome of T. timopheevi was considerably different from that of T. persicum. This genome was designated as G, emphasizing its separate identity. Instead of having the genome symbols AABB common to the Emmer group, T. timopheevi carries, according to Lilienfeld and Kihara, the genome formula AAGG.

Kostoff (1936) repeating Lilienfeld and Kihara's crosses, could only confirm their results. He disagreed, however, with their suggestion that T. timopheevi's second genome be considered as a separate entity. By the rather high chromosome pairing at meiosis of the hybrid he suspected that some homology existed between the chromosomes of the B- and G-genome. To indicate this partial homology he proposed that the second genome be designated with the symbol β, which would then give T. timopheevi the genome formula AA$\beta\beta$.

Although Love (1941) indicated that the second genome of T. timopheevi probably differed from the other 28-chromosome wheats in degree only, the genome formula (AAGG) of Lilienfeld and Kihara (1934) was generally accepted. It was suggested that the A-genome of T. timopheevi originated from the same diploid wheat species as did the A of the Emmer group. The B- and G-genomes, however, probably originated from different species (Sears, 1947).

In 1953 Sachs again studied the problem of the origin of the G-genome. T. timopheevi was crossed with other tetraploid wheat species, including T. durum (F_1 average, 9.6 bivalents), T. dicoccoides var. Kotschyanum (F_1, 10.0 bivalents) and T. dicoccoides var. nudiglumis (F_1, 13.9 bivalents). The latter hybrid displayed a remarkably regular meiosis but was almost completely sterile. This, he assumed, was due to cryptic structural hybridity. This hybrid thus showed exactly the same phenomenon as reported by Svetozarova (1939) in the hybrid between T. timopheevi and T. armeniacum. Sachs further doubled the chromosome number of this hybrid (2n = 56) and the fertility was restored.

It seemed justifiable to Sachs not to recognize T. dicoccoides var. nudiglumis as a different species. He further emphasized the fact that the basic idiogram of the chromosomes of T. timopheevi is the same as that of the other tetraploid species belonging to the Emmer group. He suggested, therefore, that the second genome of T. timopheevi and the B-genome of the Emmer species must have a common origin. He did not completely rule out the possibility of a genic effect on the meiotic behaviour in the hybrid between T. timopheevi and T. durum or T. dicoccoides var. kotschyanum. Still he favoured the assumption of structural differences in the chromosomes of the two species, since fairly regular pairing in meiosis and good fertility was observed in the artificial amphiploids of the hybrids.

He now thought that within the species T. dicoccoides a morphological as well as a chromosomal differentiation has taken place: " . . . from the Syrio-palestinicum types, such as T. dicoccoides var. kotschyanum to the cytologically differentiated types as T. dicoccoides var. nudiglumis and T. timopheevi." The origin of all the tetraploid wheats including T. timopheevi from a common 28 chromosome prototype seemed quite probable to him.

There are a few weaknesses in Sach's theory. In the assumed process of chromosome differentiation only the B-genome seemed to be involved, whereas the A-genome kept its identity. This seems highly improbable. More logical would be a theory of repeated formation of tetraploid wheat species from two similar parental species in one of which the chromosomes had been differentiated.

Another possibility is that the cause of the irregular meiosis may be due to a genic asynaptic or desynaptic effect. Such genic effect may, for example, decrease the number of chiasmata on the paired homologous chromosomes.

Studies on the G-genome of T. timopheevi were started to determine whether or not such an asynaptic gene system is operating in its hybrids. Since some fertility was observed in these hybrids, it was hoped to find a segregation of the F_2 or backcross generations for meiotic behaviour. Crosses were also made between the amphiploid, T. timopheevi x Ae. squarrosa (2n = 42, AAGGDD), and T. aestivum var. Chinese Spring, monosomic lines I to XIV (2n = 41, AABBDD). It was hoped that some indication of a genically induced asynaptic effect would be obtained in these hybrids.

In this thesis, however, only the investigations done on the hybrids of T. timopheevi and two Emmer species (T. durum and T. dicoccoides) will be treated.

B. MATERIALS AND METHODS

The following species were used, and were obtained from the sources indicated:

T. timopheevi (1) - Dr. L.P.V. Johnson (from Syria)

T. timopheevi (2) - Dr. E. R. Sears

T. durum var. *leucomelan* - University of Alberta

T. dicoccoides - Dr. E. R. Sears

The cytological methods were the same as those described in Part I of this thesis (page 14).

C. CYTOLOGICAL OBSERVATIONS OF THE F_1 HYBRIDS

1. *T. timopheevi* x *T. durum* var. *leucomelan*

Crosses between *T. timopheevi* (1) x *T. durum* var. *leucomelan* were made in the summer, 1956. Five F_1 plants were sown in the greenhouse in February, 1957. These hybrids yielded 6 F_2 seeds.

Cytology

The metaphase I of meiosis of the hybrid was studied in 387 cells. All cells showed invariably 28 chromosomes, although some giant cells with supernumary chromosomes were observed (Fig. 56). A range from cells with 6 bivalents and 16 univalents to cells with 13 bivalents and 2 univalents were observed (Table V). Multiple associations, trivalents, quadrivalents, etc., were observed in 277 cells (71.6%) of which 268 cells (69.3%) showed one or more bivalents. One trivalent was found in 214 cells (55.3%) of the metaphase cells and had almost always  form. In very few cells  , and were observed.

Several cells showed more than one multivalent. Thirty-eight (9.8%) cells showed 2 trivalents, 6 (1.6%) had three trivalents. Eleven cells (2.8%) had one trivalent and one quadrivalent, 4 (1.1%) had one quadrivalent, 3 (0.8%) had two quadrivalents. Only 2 cells (0.6%) showed chains of five and six chromosomes respectively. All of the observed quadrivalents were chains; no rings of four chromosomes were observed in this material. The most common cell type had 9 bivalents, one trivalent and seven univalents (72 cells, 18.6%). The

Table V. Chromosome associations and their frequencies in the hybrid, T. durum var. leucomelan x T. timopheevi (1)

Chromosome associations at metaphase I						No. of cells	%	Bivalents		Chiasma /biv.
VI	V	IV	III	II	I			Open	Closed	
				13	2	3	0.78	-	-	-
				12	4	12	3.10	7.17*	4.83*	1.40*
			1	12	1	1	0.26	-	-	-
				11	6	29	7.49	6.21	4.79	1.44
			1	11	3	21	5.43	5.76	5.24	1.48
				10	8	35	9.04	5.17	4.83	1.48
			1	10	5	45	11.63	5.16	4.84	1.48
			2	10	2	2	0.52	-	-	-
		1		10	4	2	0.52	-	-	-
				9	10	17	4.39	5.12*	3.88*	1.43*
			1	9	7	72	18.60	4.65	4.35	1.48
		1		9	6	2	0.52	-	-	-
			2	9	4	6	1.55	3.33*	5.67*	-
		1	1	9	3	2	0.52	-	-	-
			3	9	1	1	0.26	-	-	-
				8	12	12	3.10	4.92*	3.08*	1.39*
			1	8	9	40	10.34	4.00	4.00	1.50
		1		8	8	1	6.26	-	-	-
	1			8	7	1	0.26	-	-	-
			2	8	6	12	3.10	3.50*	4.50*	1.56*
		1	1	8	5	1	0.26	-	-	-
			3	8	3	1	0.26	-	-	-
				7	14	2	0.52	-	-	-
			1	7	11	19	4.91	3.37*	3.63*	1.52*
		1		7	10	1	0.26	-	-	-
			2	7	8	15	3.88	3.27*	3.73*	1.53*
		1	1	7	7	3	0.78	-	-	-
	1		1	7	6	1	0.26	-	-	-
			3	7	5	3	0.78	-	-	-
1		1		7	4	1	0.26	-	-	-
				6	16	1	0.26	-	-	-
			1	6	13	12	3.10	2.67*	3.33*	1.56*
			2	6	10	3	0.78	-	-	-
		1	1	6	9	1	0.26	-	-	-
			1	5	15	3	0.78	-	-	-
		1	1	5	11	2	0.52	-	-	-
			3	5	9	1	0.26	-	-	-
			1	4	17	1	0.26	-	-	-
Total						387	100.06			
Av.	0.005	0.005	0.04	0.82	8.99	7.35		4.75	4.24	1.48

* less than 20 cells

average for the 387 cells is 7.35 univalents, 8.99 bivalents, 0.82 trivalents, 0.04 quadrivalents, 0.005 pentavalents and 0.005 hexavalents.

In the last three columns of Table V the average number of open- and closed bivalents and the number of chiasmata per bivalent are shown. The number of open bivalents per cell type decreases as the number of bivalents decreases, as one might expect. The same trend is indicated in the column for the closed bivalents. It is interesting that in the last column chiasmata per bivalent remain constant. The frequencies marked with an asterisk, showing the greatest deviation, were obtained from less than 20 cells. The average of all cell types is 1.48 chiasmata per bivalent. Such a constancy of chiasmata per bivalent was also noticed in the H. jubatum x S. cereale hybrid (see Part I, pages 16 and 17).

Anaphase I showed a highly irregular distribution of chromosomes towards the poles (Table VI). The number of laggards (including those observed in telophase I) varied from 0 to 9 in 27 cells. Bridges were seldom observed in this material. In these 27 cells one bridge was noticed in a cell with a 14 - 14 distribution.

In P.M.C's of the cross T. durum var. obscurum x T. timopheevi, kindly provided by Dr. J. Kuspira and analyzed by Mr. K. Kasha, the metaphase I showed a different picture, as shown in Table VII. The lower bivalent frequency in this F_1 is striking. Averages of approximately 7 bivalents and 14 univalents were calculated. Not a single trivalent was observed in 61 cells. Apparently varietal differences in T. durum had an effect on the chromosome association in hybrids with T. timopheevi.

Table VI. Anaphase I and telophase I cells observed in the hybrid T. timopheevi (1) x T. durum var. leucomelan

Anaphase I				Telophase I	
Distribution to two poles		Laggards	Frequency	Laggards	Frequency
14	14	-	2	1	1
13	14	1	2	2	4
13	13	2	2	3	4
12	14	2	2	4	1
12	13	3	3	5	1
11	13	4	1	6	1
11	12	5	1	9	1
10	12	6	1		
		Total	14 cells		13 cells

Table VII. Chromosome associationsand their frequencies in the hybrid of T. durum var. obscurum x T. timopheevi (1)

Chromosome associations		Frequencies
I	II	No. cell
10	9	3
12	8	19
14	7	27
16	6	9
18	5	3
		61
Av. 13.67	7.16	

2. T. dicoccoides x T. timopheevi (2)

This cross was made in the greenhouse in the spring of 1957. Five F_1 seeds were sown rather late in the field (June 1, 1957). These hybrids produced many tillers. Because of frost danger (September 15, 1957) all heads had to be harvested, although several were still immature. In determining the fertility after open-pollination, 3572 florets were checked, of which 143 florets or 4.0% showed seedset or initial caryopsis development. There was considerable variation among the F_1 plants. Plants 1, 2, 3, 4 and 5 showed respectively 5.00%, 4.25%, 3.91%, 3.62% and 2.98% fertility. Twenty-one heads harvested from all five plants showed 4.14% fertility.

The cytological data from each of the five F_1 plants were very similar and therefore consolidated and treated as if obtained from one hybrid plant. Table VIII gives the chromosome associations observed at metaphase I of these hybrids (total 641 cells). The range observed varied from 2 trivalents, 3 bivalents and 16 univalents per cell to 14 bivalents per cell. The mode of this range lies near the cell types with 1 trivalent, 10 bivalents and 5 univalents (100 cells) and 1 trivalent, 9 bivalents, 7 univalents (119 cells) (Fig. 50 - 55).

The cell type with 1 trivalent, 8 bivalents, 9 univalents showed also a fairly high frequency with 58 cells. Configurations containing only bivalents and univalents (Fig. 53, 54) were observed in 153 cells (23.9%). Configurations with one trivalent (Fig. 50) had a frequency of 360 cells (56.2%); those with two trivalents (Fig. 51, 52), 91 cells (14.2%); and others with three trivalents, 9 cells (1.4%). A

Table VIII. Chromosome associations and their frequencies in the hybrid, T. timopheevi x T. dicoccoides

Chromosome associations				Frequencies		Bivalents		Chiasmata per bivalent
IV	III	II	I	No. of cells	per 100 cells	Open	Closed	
		14		1	.16			
		13	2	3	.47			
		12	4	24	3.74	5.86	6.14	1.51
	1	12	1	4	.62			
		11	6	36	5.62	5.30	5.70	1.52
	1	11	3	44	6.86	5.23	5.67	1.51
1		11	2	2	.31			
		10	8	30	4.68	5.26	4.74	1.47
	1	10	5	100	15.60	5.21	4.79	1.48
1		10	4	3	.47			
	2	10	2	9	1.40	5.33*	4.67*	1.47*
1	1	10	1	2	0.31			
		9	10	28	4.37	5.00	4.00	1.44
	1	9	7	119	18.56	4.74	4.26	1.47
1		9	6	2	.31			
	2	9	4	22	3.43	4.60	4.40	1.49
1	1	9	3	5	0.78			
		8	12	20	3.12	4.42	3.58	1.45
	1	8	9	58	9.05	4.14	3.86	1.48
1		8	8	2	.31			
	2	8	6	29	4.52	4.45	3.53	1.44
1	1	8	5	4	0.62			
	3	8	3	3	0.47			
		7	14	11	1.72	4.56*	2.44*	1.35*
	1	7	11	22	3.43	4.06*	2.94*	1.42*
	2	7	8	22	3.43	3.71	3.29	1.47
1	1	7	7	4	.62			
	3	7	5	2	.31			
	1	6	13	11	1.72	3.50*	2.50*	1.42*
	2	6	10	5	.78			
1	1	6	9	3	.47			
	3	6	7	2	.31			
	1	5	15	2	.31			
	2	5	12	2	.31			
	3	5	9	2	.31			
	2	4	14	1	.16			
1	1	4	13	1	.16			
	2	3	16	1	.16			
Total 28	588	5837	4398	641	99.98			
Av. .04	.92	9.11	6.86			4.86	4.25	1.47

* Less than 20 cells in a count.

single quadrivalent was observed in 9 cells (1.4%), and a quadrivalent together with a trivalent in 19 cells (3%). This means that in 76.1% of the 641 cells studied one or more multivalents were observed, of which 74.7% showed one or more trivalents.

The great majority of the trivalents (approx. 90%) showed the  shape (Fig. 50, 51, 52, 55), as was previously found to be true of the hybrid T. timopheevi x T. durum var. leucomelan. Although trivalents with  and  shape were rare, however approximately 12% showed the  form. The quadrivalents were all chains (Fig. 55). The average frequencies per cell were calculated to be 0.04 quadrivalents, 0.92 trivalents, 9.11 bivalents and 6.86 univalents.

In the last three columns of Table VIII (open and closed bivalents, and chiasmata per bivalent), the same trends as in Table IV (T. durum var. leucomelan x T. timopheevi) were observed. Again, the number of chiasmata per bivalent was remarkably stable for each cell type and had an overall average of 1.47. This value is amazingly close to that calculated for T. durum x T. timopheevi (1.48). All observations on both hybrids show strikingly similar chromosomal behaviour. The pairing behaviour appears thus to be identical in these two hybrids.

From the very frequent occurrence of at least one trivalent per cell, one may assume that one of the species carried a translocation that the other does not possess. In the hybrid, T. durum var. obscurum x T. timopheevi (Table VI), in which no trivalents were observed, apparently no such major translocation exists, or the same translocation exists in both species.

Figure 71 illustrates diagrammatically how such a trivalent may be produced. Let us assume that T. dicoccoides has undergone a translocation involving a large part of one chromosome (A) and a very small part of another (B); one very small and one very large chromosome are produced. The hybrid T. dicoccoides x T. timopheevi would then display a theoretical pairing complex at prophase as shown in Fig. 71 (1); owing to the small size of one chromosome it may fail to pair with the other chromosomes of the complex. The result will be a trivalent and a small univalent (Fig. 71 (2)). Frequently occurring trivalents and a negligible number of quadrivalents in the hybrid T. timopheevi x T. dicoccoides support this. Figure 71 also illustrates the two main types of trivalents found: type I () which has a frequency of 88% in cells with trivalents and type II which occurs only in 10% of these cells.

It may be of interest here to speculate, (if such a translocation exists), on what may be expected in the F_2 generation of these hybrids. The trivalent of type II will probably lead to a duplication or deficiency of a major part of a chromosome (in the accessional gamete with normal chromosome complements). Generally it is assumed that such a condition would lead to non-viability of the gamete. The trivalent of type I will give functional gametes, in gametes with normal chromosome complements. The chromosomal condition of these gametes is also illustrated in Fig. 71. In the F_2 generation, by chance fertilization of these gametes, one may expect a segregation of plants with or without trivalents in a 1:1 ratio. It may be pointed out, in passing, that this segregation will only be possible if structural

dissimilations in the chromosome are small enough to allow gametes, as illustrated in Fig. 71, to be functional. This trivalent segregation has indeed been found in the F_2 of the hybrids (Table XIX).

The behaviour of the chromosomes in the anaphase I and telophase I is shown in Table IX. The highly irregular behaviour and distribution of chromosomes towards the poles is clearly illustrated in this table. Laggards, up to 12 (Fig. 57, 58, 59), were present in 95.5% of these cells.

Bridges were observed in only 12 of the 379 cells. Note that several poles received 14 chromosomes, the normal number for these gametes.

In the interphase and tetrad stages many micronuclei were present. Several pentads were observed.

In the interphase many micronuclei were present. Apparently, as soon as the chromosomes, originated from bivalents, trivalents, etc., reach the poles (telophase I), the spindle disappears and the chromosomes in the poles begin to come into the resting stage. This process seems to go so quickly that many lagging chromosomes, split into their chromatids, fail to reach the poles in time to be included in the newly formed nuclei.

Many micronuclei were also observed in the tetrad stage. Here several pentads (Fig. 63) were noticed, in which one of the components was a very small cell (microcyte). It was observed that most of these microcytes originated at interphase (Fig. 60) giving usually two large cells with an additional small one at the top or bottom.

Table IX. Chromosome distribution to poles, and laggards observed at anaphase I and telophase I in the hybrid, T. timopheevi x T. dicoccoides

Anaphase I					Telophase I		
Chromosome distribution to pole		Lagging chromosomes	Frequency		Lagging chromosomes	Frequency	
1	2		No. of cells	Per 100 cells		No. of cells	Per 100 cells
14	14	-	2	1.40	0	8	3.40
13	15	-	7	4.86	1	21	8.94
11	17	-	1	0.69	2	37	15.74
13	14	1	14	9.72	3	60	25.53
12	15	1	2	1.40	4	39	16.60
11	16	1	1	0.69	5	22	9.36
13	13	2	8	5.56	6	23	9.79
12	14	2	11	7.64	7	13	5.53
12	13	3	18	12.50	8	5	2.13
11	14	3	1	0.69	9	3	1.28
10	15	3	1	0.69	10	2	0.85
12	12	4	17	11.81	11	-	
11	13	4	9	6.25	12	2	0.85
10	14	4	1	0.69			
11	12	4	13	9.03			
10	13	5	1	0.69	Total	235	100.00 cells
9	14	5	1	0.69			
11	11	6	4	2.78			
10	12	6	8	5.56			
9	13	6	1	0.69			
10	11	7	10	6.94			
9	12	7	1	0.69			
10	10	8	6	4.17			
9	11	8	2	1.40			
8	12	8	2	1.40			
9	10	9	1	0.69			
9	9	10	1	0.69			
		Total	144	100.00 cells			

Further, it was observed that if the two large cells went into the metaphase II stage these microcytes also showed divisions (Fig. 61, 62) of one or two chromosomes, with definite polar attraction. Later on, in telophase II and the tetrad stage, these divided chromosomes united again to form one small nucleus. It has not been observed that these microcytes again form smaller cells.

It is now believed that only whole univalents, unsplit in metaphase I because they probably fell outside the spindle action, were able to form these microcytes in the interphase. Univalents, already split in metaphase I but unable to reach the pole in time to be included in the new nucleus, probably remained as micronuclei in the cytoplasm of the large cells, since such divisions as observed in the microcytes were never seen in micronuclei scattered in the cytoplasm. The whole chromosome(s) in the microcytes then divide(s) into its (their) chromatid(s), when the two large mother cells are in metaphase II and telophase II. It is now assumed that, at least in this material, pentads cannot be formed after the second meiotic division. These abnormal cells are therefore believed to originate during anaphase I.

Plate VII. All cells are of the hybrid T. dicoccoides x T. timopheevi except the one in Fig. 57, which is from the F_1 T. timopheevi x T. durum.

Fig. 50. One trivalent, four bivalents and 17 univalents.

Fig. 51. Two trivalents, seven bivalents and eight univalents.

Fig. 52. Two bivalents (one  and one ), seven bivalents and eight univalents.

Fig. 53. Seven bivalents and 14 univalents.

Fig. 54. Eight open bivalents and 12 univalents.

Fig. 55. One quadrivalent, two trivalents, six bivalents and six univalents.

Fig. 56. Giant cell (M. I) with approximately 56 chromosomes.

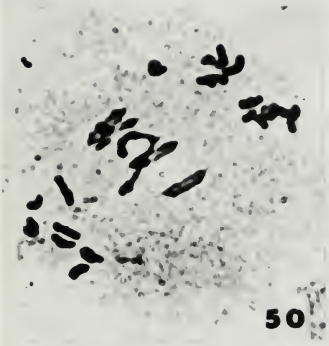
Fig. 57. Anaphase I cell with three split laggards.

Fig. 58. Late anaphase I cell with eight split laggards.

Fig. 59. Telophase I cell with 12 split laggards.

Fig. 60. Interphase diad with microcyte.

Fig. 61. Diad in metaphase II; chromosomes in microcyte also dividing.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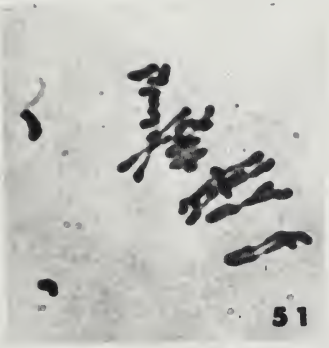
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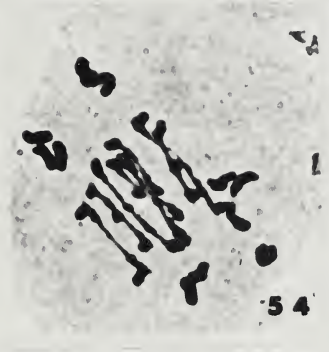
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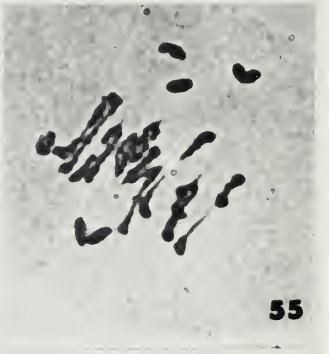
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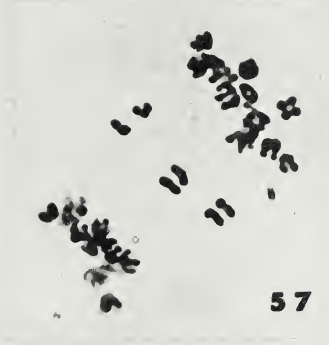
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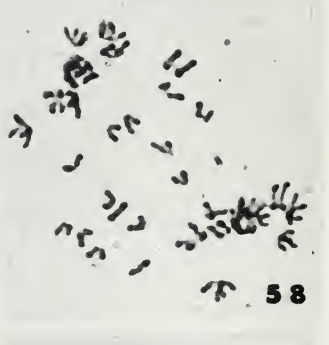
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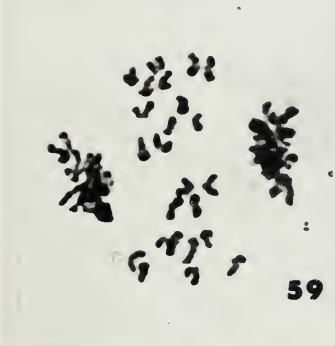
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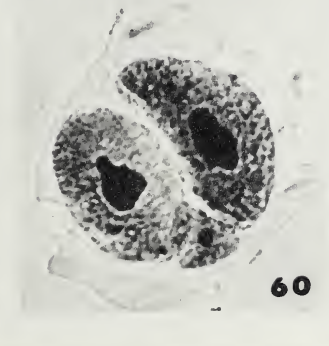
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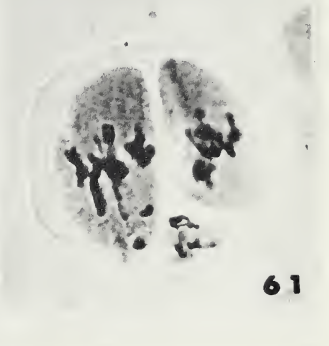

61

Plate VIII

Fig. 62. Telophase II, also in microcyte (F_1, T. dicoccoides x T. timopheevi).

Fig. 63. Pentad; microcyte at 5 o'clock, the nucleus still showing a double structure (F_1 T. dicoccoides x T. timopheevi).

Fig. 64. Metaphase I cell of F_2 plant (no. 6, 2n = 28) of hybrid, T. timopheevi x T. durum var. leucomelan, with 14 bivalents.

Fig. 65. Same plant as in Fig. 64. Metaphase I with 13 bivalents and two univalents.

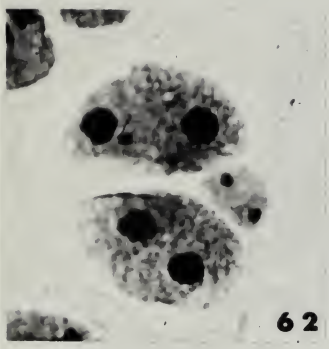
Fig. 66. Metaphase I cell of F_2 plant (no. 2, 2n = 29) of same hybrid as in Fig. 64 with 11 bivalents and seven univalents.

Fig. 67. Metaphase I cell (of plant no. 2-1, 2n = 36, of the offspring of the hybrid T. dicoccoides x T. timopheevi) with 14 bivalents and eight univalents.

Fig. 68. Metaphase I cell (of plant no. 5-1, 2n = 36, of same offspring as in Fig. 67) with nine bivalents and 18 univalents.

Fig. 69. Metaphase I cell of hybrid, H. jubatum x H. bulbosum, with four bivalents and 19 univalents (27 chromosomes).

Fig. 70. Metaphase I cell of the same hybrid as in Fig. 69 with 27 univalents.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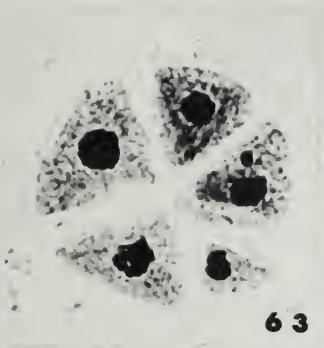
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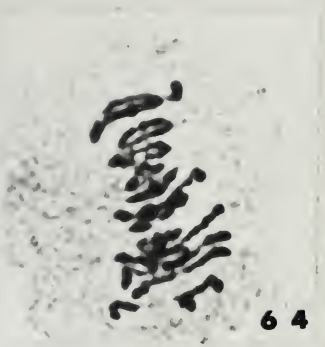
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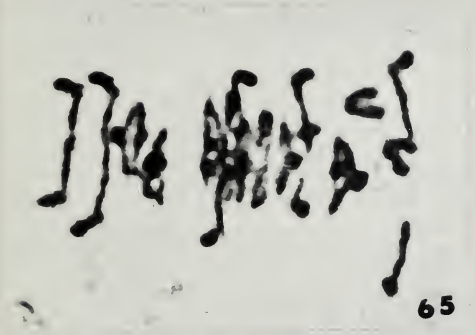
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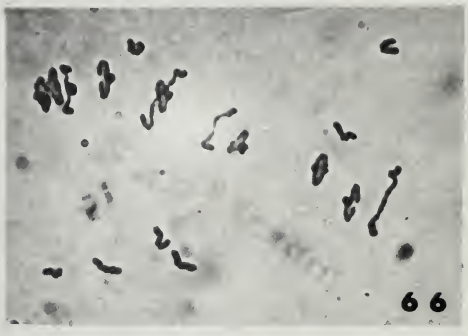
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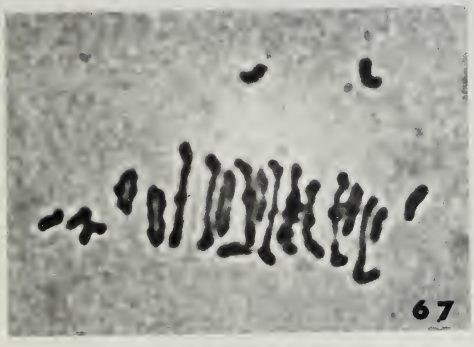
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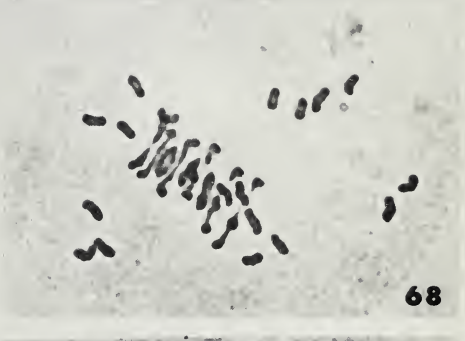
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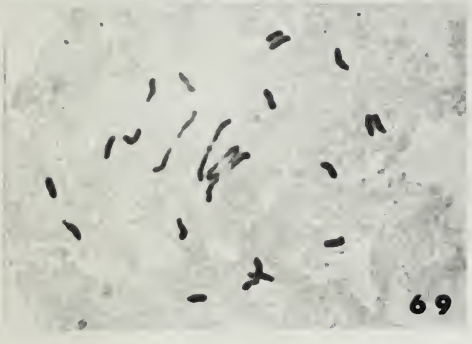
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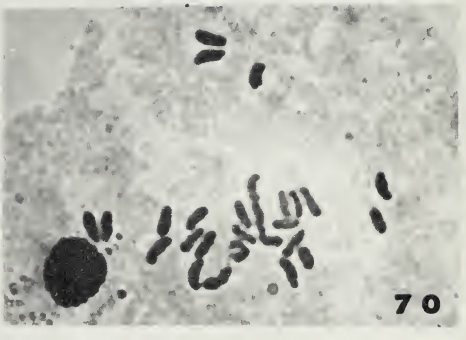

70

Plate IX

Fig. 71. Diagrammatic explanation of the observed trivalent formation in the hybrids of T. timopheevi and segregation in the F_2 for plants with and without trivalent formation.

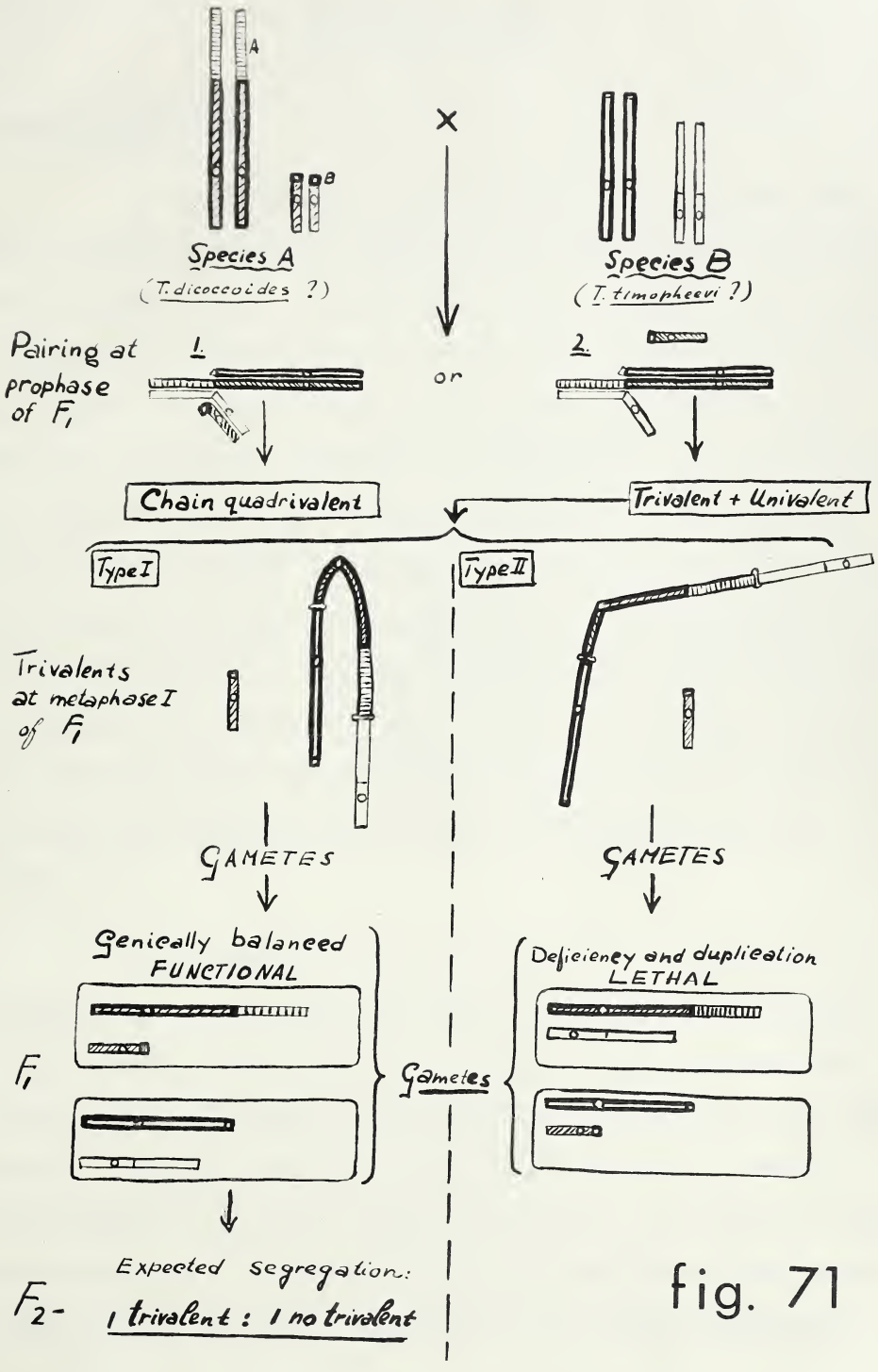

A
B
Species A
(T. dicoccoides ?)
X
Species B
(T. timopheevi ?)
Pairing at prophase of F1
1.
or
2.
Chain quadrivalent
Trivalent + Univalent
Type I
Type II
Trivalents at metaphase I of F1
GAMETES
GAMETES
Genically balanced
FUNCTIONAL
Deficiency and duplication
LETHAL
F1
Gametes
Expected segregation:
F2 - 1 trivalent : 1 no trivalent

fig. 71

D. ANALYSIS OF F_1 DATA

Causes of sterility

In the introduction the possibility of an asynaptic effect in the hybrids involving T. timopheevi and Emmer species was advanced. Such a genetic system, if operating in these tetraploid wheat hybrids, would induce a highly irregular meiosis. Despite this, some fertility may still be expected to occur in these hybrids according to this asynapsis hypothesis. Therefore the chromosomes of the B- and G-genome may then be quite similar in chromosomal and genic make-up and the sterility may be caused exclusively by the irregular behaviour of the chromosomes at meiosis. Random distribution of univalents towards the poles and occurrence of laggards at anaphase I will lead to completely unbalanced, non-functional gametes. However, by this chance distribution one might expect the occasional formation of functional gametes, in which each chromosome and its genes are represented once and only once.

It is generally recognized that two kinds of sterility are distinguishable: chromosomal and genic (Dobzhanski, 1937; Thompson, 1940; Stebbins, 1950). Genic sterility may be induced by gene mutations which may disturb the gametogenesis or the fertility of the offspring. Asynaptic genes, for example, cause an irregular meiosis which results in (partial) sterility (Fig. 72). The sterility is then not a result of dissimilarities in the gross structure of the chromosomes, but of the genetic constitution of the organism (Dobzhanski,

1937). Chromosomal sterility will induce an irregular meiosis in a hybrid, because of lack of homology between the chromosomes. This will lead to unbalanced chromosome and gene complements in the gametes.

The sterility strictly due to a gene-induced asynaptic or desynaptic effect - as is suggested possibly to be operating in the T. timopheevi hybrids - may then be placed in the large group of sterility phenomena called genic sterility. But if there are also some structural effects controlling the bivalent formation in such a hybrid, then the sterility may also be classed as chromosomal. In both cases gametes will be formed with abnormal chromosomal constitutions, such as too many and too few chromosomes, and duplications together with deficiencies of entire chromosomes. These gametes will always be non-functional, regardless of what the causes of the irregular meiosis (the immediate cause of these abnormal gametes) may be. It is essential in the present studies to recognize this effect as a separate source of sterility and to ignore deliberately the cause of the irregular meiosis. For convenient reference the name "divisional effect" is, therefore, suggested. It is thought, then, that chromosomal sterility, in a stricter sence, operates only in gametes with complete chromosome sets. Structural differences in these chromosomes will give rise to non-functional gametes ("chromosomal-effect," Fig. 72).

The total sterility (S) in a hybrid can now be expressed in the following formula:

$$S = d + c + G$$

in which d refers to the divisional effect, c to the chromosomal effect and G to a genic effect, which may operate together with the chromosomal effect. This formula may be applicable to any hybrid. It is, however,

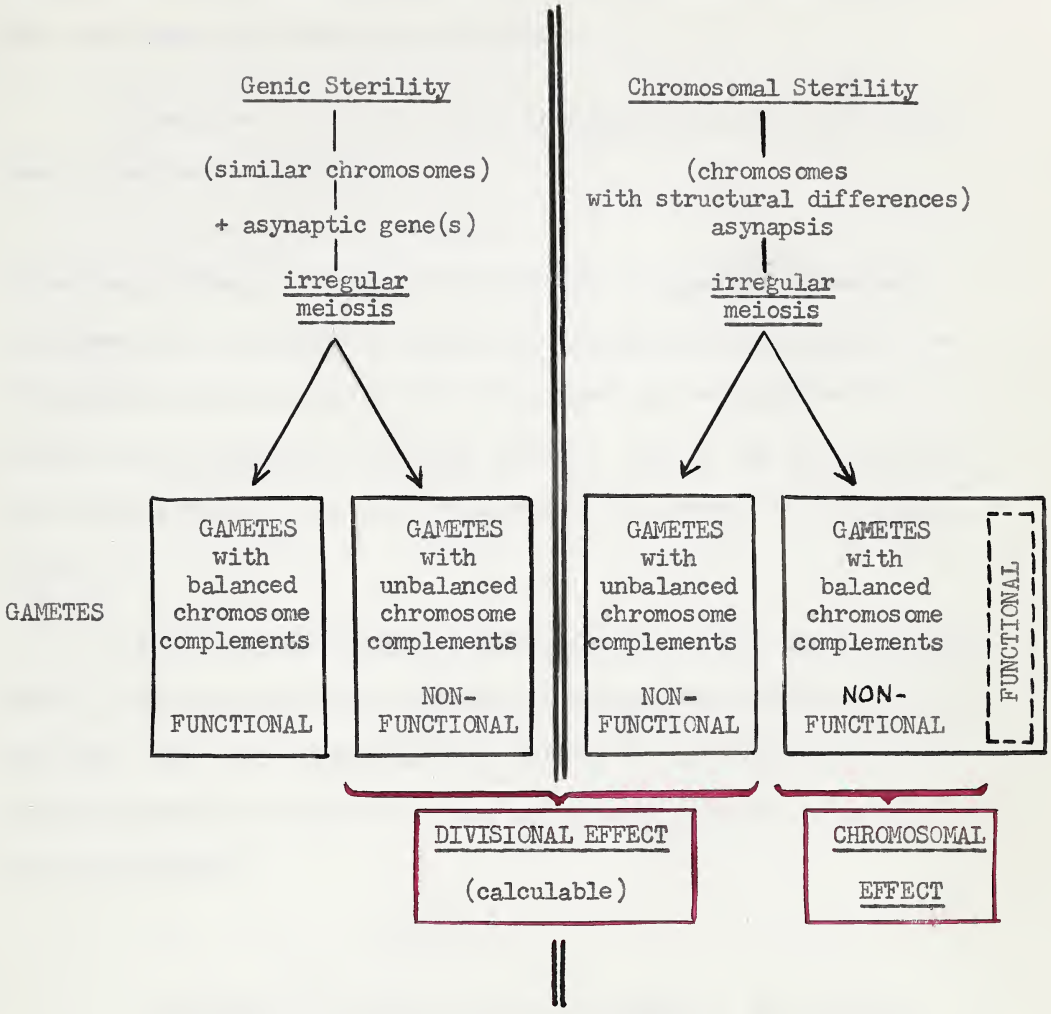


Fig. 72. Diagram illustrating the concept of Divisional- and Chromosomal Effect operating in the gametes of plants with irregular meiosis. In black the two kinds of sterility as defined by Dobzhanski (1937, 1951): genic sterility here limited to the effect of asynaptic genes. In red Divisional and Chromosomal Effect operating in plants exhibiting genic- and chromosomal sterility.

assumed that, in interspecific hybrids, the chromosomal sterility is of more importance than the genic sterility.

The above formula for these interspecific hybrids may therefore be reduced to the form:

$$S = d + c$$

Chromosomal effect, c, induces non-viability in gametes with normal chromosome sets (obtained by chance distribution of univalents). The structural dissimilarities of the chromosomes are responsible for unbalanced gene combinations in these gametes. All of the other gametes containing abnormal chromosome combinations fall under the divisional effect, d.

Now if a method could be developed by which d, the divisional effect, could be calculated, then the chromosomal effect (c) could be measured. The total plant sterility (S) may be determined as "percent sterile florets" and since d is calculated, therefore the value of c may be determined,

$$c = S - d$$

This value of c will be directly related to the existing structural differences between the chromosomes belonging to different genomes in the interspecific hybrid. The greater the structural dissimilarities are, the greater the value c (and d) will be and the more sterile the hybrid will be. If no structural differences between the chromosomes exist, then c must be zero making the formula S = d.

In such a case it may be assumed that the cause of the divisional effect (d) is a genic effect on chromosome pairing or chiasma formation, giving rise to an irregular meiosis.

In case of cryptic structural hybridity, as in the hybrid of T. timopheevi and T. dicoccoides var. nudiglumis (Sachs, 1953; see pages 38 and 41) the formula will be

$$S = c$$

Here bivalent formation is normal. Small structural dissimilarities in the chromosomes, however, cause formation of genically unbalanced gametes. These gametes will be non-functional, despite the normal meiosis.

Another genic effect on viability of the gametes is, of course, also quite possible in the cryptic structural hybrid. It is emphasized here, that all combinations are possible. In explaining the theory all examples and formulas are kept simple in order to demonstrate the operation of the basic sterility effects. In the evaluation of results obtained from studies on such hybrids all combinations of effects and complications of other effects must be considered before conclusions can be drawn.

The following pages describe an attempt to evaluate the influence of the divisional effect on the fertility of the hybrid plant. These calculations were undertaken with the expectation that they might provide a basis for judging the possible relationship of the G-genome of T. timopheevi and the B-genome of the Emmer wheat species.

Basic assumptions for calculations

For the calculation of the divisional effect in the hybrid T. dicoccoides x T. timopheevi six assumptions have to be made, as follows: (1) T. dicoccoides and T. timopheevi are assumed (for the purpose of

calculations only) to have identical genomes. The irregular meiosis is accepted without questioning the cause. The sterility of the hybrid will then be entirely due to the divisional effect; thus S = d. If the hybrid sterility is greater than the calculated divisional effect, then another effect, such as a chromosomal effect, must also be present (see assumption 6).

(2) The fertility of the hybrid is entirely governed by the female gametes. In the hybrids discussed here, anthers often fail to dehisce. If no other source of pollination is available the occasional functional egg cell will not always be fertilized. If there is a good pollination source then seed set will give a good index of functional female gametes formed by the hybrid.

(3) The chromosomal behaviour at meiosis is the same in the ovaries and the anthers. This will mean that conclusions drawn on the pollen mother cells can also be extended to the embryo sac mother cells.

(4) In metaphase I, anaphase I and telophase I of the hybrids, behaviour of the univalents is completely random. Univalents will move randomly to the poles, or will lag, splitting as they do so in a random fashion in the anaphase I stage. This random movement of univalents has been a source of dispute in the past. However, in the present investigation random movement had to be assumed, since calculations could be made only on that basis. This randomness will be verified later.

(5) Chromosomes that lag and split at anaphase I are always eliminated from the gametic nucleus during anaphase I and anaphase II. These chromosomes become micronuclei, which are supposed to degenerate in the cytoplasm.

(6) Since it is assumed that the two parental species have common

genomes, all gametes with normal chromosome complements (14 chromosomes, in which each chromosome and each gene is represented once and only once) are considered to be functional in the hybrid. Gametes not having the exact haploid complement of the hybrid are non-functional. Such gametes comprise those with less or more than 14 chromosomes, and those with 14 chromosomes in which deficiencies of some types are made up by duplication of others. This assumption is not entirely justified, since, in the tetraploid wheats, plants are occasionally found that have arisen from 15-chromosome gametes. Gametes with 13 chromosomes or less are, however, non-functional. For the calculation the assumption of viable gametes with 14 chromosomes is admittedly temporary.

Outline of procedure

For these calculations, data of the hybrid T. dicoccoides x T. timopheevi, as presented in Tables V and VI, were used. All calculations were made on observations of metaphase I (641 cells). From these data the theoretical hybrid fertility, chromosome distribution and lagging at anaphase I were computed. The theoretical hybrid fertility was then compared with the observed hybrid fertility. The expected chromosome distributions to the poles and lagging at the equatorial plate, thus obtained, were verified by observations of anaphase I (number of chromosomes at the poles and laggards) and telophase I (only laggards). This last stage was included since relatively few anaphase I cells (144) could be found. The telophase I cells allowed a clear observation of lagging chromosomes, and increased the sample by 235 cells.

Calculation of the divisional effect

The scoring of metaphase I cells was kept as simple as possible. Cell types with their chromosome associations, type and nature of associations (open, close bivalents, types of trivalents, quadrivalents, etc.) were scored. In the case of univalents, only the number was scored, no attention being given to position in the metaphase plate or at the poles. These observations were omitted since it was thought that if all univalent movement was random, positions at the metaphase plate and poles would also be a random matter. Side-by-side associations, which were found to have a positive effect on the chromosome movement in haploid wheat (Person, 1956) were seldom observed. These were, therefore, also omitted in the scoring. End-to-end associations occurred more frequently. However, they have probably no effect on chromosome behaviour (Person, 1956).

The chromosomal make-up of each pole is considered basic for the gametes it will produce. Split chromosomes at the pole will be removed from the nucleus in anaphase II and telophase II where they will lag behind, failing to be included in the nucleus of the new gamete. The constitutions of unsplit chromosomes at the poles were considered as the constitution of one gamete, although in reality each pole produces two identical gametes. This was done to avoid unnecessarily large numbers in the calculations. The results will be the same in both cases.

In Table VII all cell types and their frequencies, as recorded for 641 cells, are listed. The range is from cells with 14 bivalents without univalents to cells with 3 bivalents, 2 trivalents and 16 univalents.

For each of these cell types the chances were calculated for the formation of gametes with 14 chromosomes in which each chromosome is only once represented (hereafter referred to as "normal gametes"). Every chromosome in the hybrid is considered to have an identical homologue in the sense that either of the two may replace the other in the gamete without disturbing its ability to function. The cell type with 14 bivalents will give only normally functional gametes and is therefore given the "viable fraction" of 1. For the cell type having 13 bivalents, 2 univalents, the situation is somewhat more involved.

The 13 bivalents will split into 13 chromosomes at each pole. The two univalents may contribute 0, 1 or 2 extra chromosomes to either pole. Since randomness is assumed this distribution will be 1(0) : 2(1) : 1(2) thus giving gametes with 13, 14 or 15 chromosomes in this ratio. Lagging is also involved. Of the two univalents, 0, 1 or 2 may lag per cell. This lagging will also occur in a 1(0) : 2(1) : 1(2) ratio, since there are two univalents involved. Table X shows how the total number of gametes and the viable combinations were determined for a cell with 13 bivalents and two univalents. Twelve functional gametes out of the possible 32 gametes were found in this way. This cell type, therefore, has a "viable fraction" of 12/32 or 3/8.

The same was done for the next cell type, 12 bivalents, 4 univalents. The distribution ratio of the four univalents towards the poles is 1(4 to one pole - 0 to the other) : 4(3 - 1) : 6 (2 - 2) : 4(1 - 3) : 1(0 - 4). The lagging ratio is 1(0) : 4(1) : 6(2) : 4(3) : 1(4). These ratios combined gave 513 gametic combinations of which 72 were viable. The viable fractions for this cell type is thus 72/512 or $(3/8)^2$.

Table X. Gamete formation of a cell with 13 bivalents and 2 univalents. Univalent distribution to the poles and lagging each considered to be at random.

	1	2		1	
1	13 + 0	13 + 0	13 + 0	13 + 0	pole
		1^1	1	11^1	laggards
	13 + 11^1	[13 + 1]	[13 + 1^1]	13 + 0	pole
2	[13 + 1]	13 + 0	[13 + 1]	13 + 0	pole
		1	1^1	11^1	laggards
	[13 + 1^1]	[13 + 1^1]	13 + 0	13 + 0	pole
	[13 + 1^1]	13 + 0	[13 + 1^1]	13 + 0	pole
		1^1	1	11^1	laggards
	[13 + 1]	[13 + 1]	13 + 0	13 + 0	pole
1	13 + $1^1$1	[13 + 1^1]	[13 + 1]	13 + 0	pole
		1	1^1	$1^1$1	laggards
	13 + 0	13 + 0	13 + 0	13 + 0	pole

The numeral 1 represents a chromosome; 1^1 is the homologue of 1. The combinations enclosed by a square are assumed to be functional gametes. In this cell type (13^{II} 2^{I}) there are 12 viable gametes out of the 32 possible combinations.

Table XI shows the expected lagging of univalents at anaphase I of some other cell types, the total gametic combinations possible and the proportion of functional gametes for each. The last column gives the mathematical formula for ratios of viable gametes to total gametes. Each extra pair of univalents decreases the viable fraction by a factor of 3/8. For each trivalent present the viable fraction is decreased by a factor of 1/2. The "viable fraction" (Vf) may thus be expressed by the following formula for all cells, with or without trivalents

$$Vf = (1/2)^t \cdot (3/8)^{1/2(n-t)}$$

where t is the number of trivalents and n is the number of univalents per cell.

For each cell type observed in metaphase I of the hybrid (Table VIII), the corresponding fertility value (F) has been calculated (Table XII) by use of this formula, multiplied by the cell frequencies. In cells with quadrivalents, one quadrivalent was considered as two bivalents (an adjustment for this type of association will be discussed later). The values thus calculated for each cell type observed are then added giving the theoretical, or expected fertility value of the hybrid plants, based on observations of metaphase I cells. This value is, of course, expressed as a percentage, since the calculations were made on the percentage frequency of each cell type. The fertility value of the hybrid thus calculated gave a value of 5.819%.

This value is, in fact, the calculated chances out of 100 of the hybrid, with its irregular meiosis, forming gametes with a normal set of chromosomes. These gametes will then be functional, provided

Table XI. Ratios of lagging chromosomes, total possible gametic combinations (with random distribution of univalents to poles) and number of viable gametes.

Cell types	Lagging Ratio* No. of laggards 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total combinations	Viable combinations	Ratio
14^{II}	1															1	1	1
$13^{II}\ 2^{I}$	1	2	1													32	12	3/8
$12^{II}\ 4^{I}$	1	4	6	4	1											512	72	$(3/8)^2$
$11^{II}\ 6^{I}$	1	6	15	20	15	6	1									8,192	432	$(3/8)^3$
$10^{II}\ 8^{I}$	1	8	28	56	70	56	28	8	1							131,072	2592	$(3/8)^4$
$9^{II}\ 10^{I}$	1	10	45	120	210	252	210	120	45	10	1					2,097,152	15,552	$(3/8)^5$
$8^{II}\ 12^{I}$	1	12	66	220	495	792	924	792	495	220	66	12	1			33,554,432	93,312	$(3/8)^6$
$7^{II}\ 14^{I}$	1	14	91	364	1001	2002	3003	3432	3003	2002	1001	364	91	14	1	536,870,912	559,872	$(3/8)^7$
$1^{III}\ 12^{II}\ 1^{I}$	1	1														8	4	1/2
$1^{III}\ 11^{II}\ 3^{I}$	1	3	3	1												128	24	1/2 . 3/8
$1^{III}\ 10^{II}\ 5^{I}$	1	5	10	10	5	1										2048	144	$1/2(3/8)^2$
etc.						etc.												
																16x	6x	$(1/2)^t(3/8)^{\frac{1}{2}(n-t)}$

proportion of viable gametes (or viable fraction)

* expansion of binomial $(1/2 + 1/2)^n$ using coefficients only (n = number of univalents)

Table XII. Calculation of the theoretical fertility value of the hybrid, T. dicoccoides x T. timopheevi, using the formula $Vf=(1/2)^{t}.(3/8)^{\frac{1}{2}(n-t)}$

Cell types	Frequency in %	$Vf=(1/2)^{t}.(3/8)^{\frac{1}{2}(n-t)}$ x frequency	Fertility value	F
14^{II}	0.156	1 x 0.156	0.156	
$13^{II}\ 2^{I}$	0.47	3/8 x 0.47	0.172	
$12^{II}\ 4^{I}$	3.74	9/64 x 3.74	0.526	
$11^{II}\ 6^{I}$	5.62	27/512 x 5.62	0.296	
$10^{II}\ 8^{I}$	4.68	81/4096 x 4.68	0.093	
$9^{II}\ 10^{I}$	4.37	243/32768 x 4.37	0.032	
$8^{II}\ 12^{I}$	3.12	729/262144 x 3.12	0.009	
$7^{II}\ 14^{I}$	1.72	2187/2097152 x 1.72	0.002	
Total	23.87	Cells with only bivalents + univalents	1.286	1.286
$1^{III}\ 12^{II}\ 1^{I}$	0.62	1/2 x 0.62	0.31	
$1^{III}\ 11^{II}\ 3^{I}$	6.86	1/2 x 3/8 x 6.86	1.286	
$1^{III}\ 10^{II}\ 5^{I}$	15.60	1/2 x 9/64 x 15.60	1.096	
$1^{III}\ 9^{II}\ 7^{I}$	18.56	1/2 x 27/512 x 18.56	0.489	
$1^{III}\ 8^{II}\ 9^{I}$	9.05	1/2 x 81/4096 x 9.05	0.089	
etc.	etc.	etc.	etc.	
Total	56.16	Cells with one trivalent, etc.	3.287	3.287
	Total frequency in %			
2^{III}... ...	14.20	Cells with two trivalents, etc.		0.880
3^{III}... ...	1.40	Cells with three trivalents, etc.		0.081
1^{IV}	1.40	Cells with one quadrivalent, etc.		0.102
$1^{IV}\ 1^{III}$... ...	2.96	Cells with one quadrivalent, one trivalent, etc.		0.183
Total	99.99	Total % of viable gametes in F_1		5.819%

no structural chromosomal dissimilarities cause inviability of the gametes. The calculated sterility, due to unbalanced chromosome complements in the gametes, will then be 100 - 5.8 = 94.2%. This sterility value represents the amount of sterility caused by the irregular meiotic division of the hybrid, or, in other words, the divisional effect of the irregular meiosis.

A few adjustments have been found to be necessary. Twelve percent of the trivalents showed the  form and 88% the  configuration. It is believed that the  trivalent will lead to gametic inviability (Fig. 71), whereas the other type of trivalent will give viable gametes. The fertility value of all cells with one trivalent is 3.287%. This will be reduced by 0.394% (12% of its value). Approximately 50% of the quadrivalents showed a  configuration (gives functional gametes) and 50% had a  configuration (gives non-functional gametes). The fertility value of cells with 1 quadrivalent (0.285%) will thus be reduced by 50% and become 0.142%. There are also other cell types (with 2 trivalents, etc.) which might be taken in account for adjustments. The sterility effect of these cells will, however, be neglected here. The total value of the adjustment is thus 0.394 + 0.142 = 0.536% or approximately 0.5%. This sterility value is not a divisional effect, as defined earlier, but is strictly chromosomal. The divisional effect of 94.2% will thus be increased by 0.5% of measurable chromosomal effect, making the total calculated sterility 94.7%. The theoretical plant fertility is then 100 - 94.7 = 5.3%.

This calculated fertility value of the hybrid seems very close to the observed fertility value of 4.0%, provided that the basic assumptions, underlying the method of calculation, are correct.

The observed sterility of the hybrid is 100% - 4% = 96%. The sterility formula for interspecific hybrids is assumed to be:

$$S = d + c$$ (see page 58).

A slight change of this formula is necessary, since not only the divisional sterility effect is calculated, but also a part of the chromosomal effect, due to translocations. This will change the formula to:

$$S = d + c_1 + c_2$$

in which it is assumed that c_1 is the effect of the translocation on the gametic viability, and c_2 the effect of the genomic structural differences of the chromosomes, if present. Substituting the values obtained in this formula gives

$$96\% = 94.2\% + 0.5\% + c_2$$

$$c_2 = 1.3\%$$

This value of c_2 is, however, negligible, if the basic assumptions are correct.

Before any conclusions can be drawn it is necessary to verify the two most critical assumptions:

(a) The randomness of the univalent (and multivalent) behaviour at meiosis of the hybrid; and

(b) the non-viability of gametes other than the "normal" type with 14 chromosomes.

If other gametes are viable these will be detectable in the offspring of the hybrids. A separate section will be devoted to such offspring.

Verification of the assumption of random behaviour of univalents and multivalents

The verification of random behaviour of univalents at anaphase I must be based on:

(a) the lagging of the chromosomes and

(b) the chromosome distribution to the poles at anaphase I.

(a) Lagging of the univalents at anaphase I

The theoretical values for expected lagging at anaphase I, based on random behaviour of univalents observed in metaphase I, may be obtained from Table XIII. When one expresses the ratios of lagging chromosomes in terms of the expanded binomial $(\frac{1}{2} + \frac{1}{2})^n$ and multiplies the fraction by the corresponding cell frequencies (expressed in percentages), values (in %) are obtained, which indicate the theoretical lagging expected for each cell type. Table XIII and XIV indicate the calculations and show the theoretical ratios of lagging, based on the frequencies of the metaphase I cells. This ratio, expressed in percentages is for 0 to 11 lagging univalents:

3.6% (0), 11.7% (1), 19.3% (2), 21.3% (3), 17.9% (4), 12.2% (5), 7.2% (6), 3.8% (7), 1.8% (8), 0.8% (9), 0.3% (10) and 0.1% (for 11 laggards).

In Table XV these theoretical values are compared with the number of laggards observed in cells at anaphase I and telophase I. It is noteworthy that the telophase I cells show a non-significant X^2 and a correspondingly high P-value (P = .22). The anaphase I cells show a stronger lagging than that expected on the basis of random behaviour ($X^2 = 39.66$; $P < 0.01$). Although the population of anaphase I

Table XIII. Example of a part of the calculation of the theoretical lagging of univalents observed in metaphase I, assuming that lagging occurs in random fashion.

Cell types	Cell frequency in %	Lagging chromosomes										
		0	1	2	3	4	5	6	7	8		
14^{II}	0.157	1/1									x 0.157	
$13^{II}\ 2^{I}$	0.47	1/4	2/4	1/4							x 0.47	see further in
$11^{II}\ 4^{I}$	3.74	1/16	4/16	6/16	4/16	1/16					x 3.74	
$10^{II}\ 6^{I}$	5.62	1/64	6/65	15/64	20/64	15/64	6/64	1/64			x 5.62	Table XIV
$9^{II}\ 8^{I}$	4.68	1/256	8/256	28/256	56/256	70/256	56/256	28/256	8/256	1/256	x 4.68	
etc.	etc.					etc.					etc.	
$1^{III}\ 12^{II}\ 1^{I}$	0.62	1/2	1/2								x 0.62	
$1^{III}\ 11^{II}\ 1^{I}$	6.86	1/8	3/8	3/8	1/8						x 6.86	
$1^{III}\ 10^{II}\ 1^{I}$	15.60	1/32	5/32	10/32	10/32	5/32	1/32				x 15.60	
$1^{III}\ 9^{II}\ 1^{I}$	18.56	1/128	7/128	21/128	35/128	35/128	21/128	7/128	1/128		x 18.56	
etc.	etc.					etc.						

Table XIV. Last part of the calculation of the theoretical lagging of univalents observed in metaphase I, assuming that lagging occurs in random fashion (continued from Table XIII).

Cell types	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Total %
14^{II}	0.157														
$13^{II}\ 2^{I}$	0.117	0.234	0.117												
$12^{II}\ 4^{I}$	0.234	0.935	1.403	0.935	0.234										
$11^{II}\ 6^{I}$	0.088	0.527	1.317	1.756	1.317	0.527	0.088								
$10^{II}\ 8^{I}$	0.018	0.146	0.512	1.024	1.280	1.024	0.512	0.146	0.018						
$9^{II}\ 10^{I}$	0.004	0.043	0.192	0.511	0.895	1.074	0.895	0.511	0.192	0.443	0.004				
$8^{II}\ 12^{I}$	0.001	0.009	0.050	0.167	0.376	0.602	0.702	0.602	0.376	0.167	0.050	0.009	0.001		
$7^{II}\ 14^{I}$	0.000	0.001	0.009	0.038	0.104	0.208	0.312	0.356	0.312	0.208	0.104	0.038	0.009	0.001	
Total	0.619	1.895	3.600	4.413	4.206	3.435	2.509	1.615	0.898	0.418	0.158	0.047	0.010	0.001	23.84%
Similar for other cell types.															
Total for cells with															
1 trivalent:	1.821	6.516	11.238	12.632	10.452	6.828	3.677	1.752	0.765	0.306	0.106	0.031	0.007	0.001	56.13%
2 trivalents:	0.649	2.098	3.110	3.132	2.420	1.454	0.721	0.315	0.140	0.069	0.033	0.014	0.005	0.001	14.16%
3 trivalents:	0.072	0.246	0.346	0.292	0.209	0.137	0.068	0.024	0.005	0.001					1.40%
1 quadrivalent:	0.113	0.311	0.361	0.282	0.187	0.097	0.039	0.010	0.001						1.40%
1 trivalent + 1 quadrivalent:	0.278	0.587	0.623	0.543	0.395	0.260	0.141	0.069	0.032	0.014	0.005	0.001			2.95%
Total	3.552	11.653	19.278	21.312	17.869	12.211	7.155	3.785	1.841	0.808	0.302	0.093	0.022	0.003	99.88
Expected lagging in %	3.6	11.7	19.3	21.3	17.9	12.2	7.2	3.8	1.8	0.8	0.3	0.1	-	-	100.0%

Table XV. Calculation of X^2- and corresponding P-values for lagging of univalents observed in anaphase I and telophase I cells of the hybrid T. dicoccoides x T. timopheevi

No. of laggards	Anaphase I cells			Telophase I cells			Anaphase I and Telophase I cells		
	O	T	$\frac{(O-T)^2}{T}$	O	T	$\frac{(O-T)^2}{T}$	O	T	$\frac{(O-T)^2}{T}$
0	10	8.2	4.43	8	8.5	0.03	18	13.6	1.42
1	17	16.8	0.00	21	27.5	1.54	38	44.3	0.90
2	19	27.8	2.79	37	45.4	1.55	56	73.1	4.00
3	20	30.7	3.73	60	50.1	1.96	80	80.7	0.00
4	27	25.8	0.06	39	42.1	0.23	66	67.8	0.02
5	15	17.6	0.38	22	28.7	1.56	37	46.2	1.83
6	13	10.4	0.68	23	16.9	2.20	36	27.3	2.77
7	11	5.5	5.50	13	8.9	1.89	24	14.4	6.40
8	10	2.6	21.06	5	4.2	0.15	15	6.8	9.88
9	1	1.2	0.03	3	1.9	0.64	4	3.0	0.33
10	1	0.4	0.90	2	0.7	2.41	3	1.1	3.28
11	-	0.1	0.10	-	0.1	0.10	-	0.4	0.40
12	-	-	-	2*	-		2	-	
	144	X^2 = 39.66		235	X^2 = 14.26		379	X^2 = 31.23	
	D.F. = 11; P < 0.01			D.F. = 11; P = 0.22			D.F. = 11; P < 0.01		

* This class was not included in the calculations.

cells is fairly small (144 cells) it has its effect on the total population of anaphase I and telophase I cells, causing for this population a significant X^2 of 31.23 ($P < 0.01$). The question arises as to whether this slightly stronger lagging is due to sampling error in the fairly small samples, subdivided into 12 classes, making each class very small, or to a definite tendency to stronger lagging due to incomplete randomness or multivalent laggards. The author believes that it is probably due to the sampling error of the fairly small population of cells. However, the possibility of extra-univalent lagging does exist since it has been shown by other workers (Darlington, 1929, 1937; Myers, 1944a, 1944b) that multivalents may produce lagging chromosomes at anaphase I of meiosis. This stronger lagging should then also have been reflected in the telophase I cells. Here, however, the X^2 proved to be insignificant. A larger population of anaphase I and telophase I cells would probably give a better indication of the actual situation.

(b) Chromosome distribution to the poles at anaphase I

It was attempted to show how each cell type at metaphase I, and on the basis of randomness, would distribute its chromosomes towards the poles. A cell type with 14 bivalents will distribute 14 chromosomes to the two poles. A cell type with 13 bivalents and two univalents will distribute its chromosomes to the poles in a ratio of 9 (13 chromosomes) : 6 (14) : 1 (15). For the cell type with 12 bivalents and 4 univalents the distribution ratio will be 81 (12 chromosomes) : 108 (13) : 54 (14) : 12 (15) : 1 (16). This distribution apparently is an expansion of the binomial $(3/4 + 1/2)^n$ where n is the number of univalents (Table XVI). If there is a trivalent in the cell

Table XVI. Ratios of chromosome distribution to the poles at anaphase I expected for some metaphase I cell types, if chromosome behaviour is random.

	Chromosome distribution to the poles*								
	10	11	12	13	14	15	16	17	18
14^{II}					1				
$13^{II}\ 2^{I}$				9	6	1			
$12^{II}\ 4^{I}$			81	108	54	12	1		
$11^{II}\ 6^{I}$		729	1,458	1,215	540	135	18	1	
$10^{II}\ 8^{I}$	6,561	17,496	20,412	13,608	5,670	1,512	252	24	1
etc.		etc.							
$1^{III}\ 12^{II}\ 1^{I}$				3	4	1			
$1^{III}\ 11^{II}\ 3^{I}$			27	54	36	10	1		
$1^{III}\ 10^{II}\ 5^{I}$		243	648	675	360	105	16	1	
$1^{III}\ 9^{II}\ 7^{I}$	2,187	7,290	10,206	7,938	3,780	1,134	210	22	1
etc.		etc.							
$2^{III}\ 10^{II}\ 2^{I}$			9	24	22	8	1		
$2^{III}\ 9^{II}\ 4^{I}$		81	270	351	228	79	14	1	
$2^{III}\ 8^{II}\ 6^{I}$	729	2,916	4,860	4,428	2,430	828	172	20	1
etc.		etc.							
$3^{III}\ 9^{II}\ 1^{I}$			3	10	12	6	1		
$3^{III}\ 8^{II}\ 3^{I}$		27	108	171	136	57	12	1	
$3^{III}\ 7^{II}\ 5^{I}$	243	1,134	2,214	2,358	1,500	586	138	18	1
etc.		etc.							

* Expansion of $(3/4 + 1/4)^{n} \cdot (1/2 + 1/2)^{t}$ using numerators only (common denominators).
n = number of univalents
t = number of trivalents

type a second binomial is involved for the effect of the trivalent. For the cell type 12 bivalents, 1 trivalent and 1 univalent the pole ratio of 3 with 13 chromosomes, 4 with 14 chromosomes and 1 with 15 chromosomes was obtained. This ratio is derived (using numerators only) from:

$$\begin{array}{l} 3 + 1 \\ \underline{\quad 3 + 1} \\ 3 + 4 + 1 \end{array} \qquad (3/4 + 1/4)^1 . (1/2 + 1/2)^1$$

A similar computation is used for the polar distribution of the chromosomes of the cell type with 12 bivalents, 1 trivalent, 3 univalents, in which the pole ratio is 27 (12 chromosomes) : 54 (13) : 36 (14) : 10 (15) : 1 (16). This ratio is derived as follows (using numerators only):

$$\begin{array}{l} 27 + 27 + 9 + 1 \\ \underline{\quad 27 + 27 + 9 + 1} \\ 27 + 54 + 36 + 10 + 1 \end{array} \qquad (3/4 + 1/4)^3 . (1/2 + 1/2)^1$$

In cell types with two trivalents the matter becomes more complicated. The cell type with 10 bivalents, 2 trivalents and 2 univalents, shows a random pole distribution of 9 poles with 12 chromosomes, 24 poles with 13 chromosomes, 22 poles with 14 chromosomes, 8 poles with 15 chromosomes and 1 pole with 16 chromosomes. This ratio was obtained as follows (using again only the numerators):

$1(9 + 6 + 1) + 2(9 + 6 + 1) + 1(9 + 6 + 1)$ giving

$$\begin{array}{l} 9 + 6 + 1 \\ 18 + 12 + 2 \\ \underline{\quad 9 + 6 + 1} \\ 9 + 24 + 22 + 8 + 1 \end{array} \qquad (3/4 + 1/4)^2 . (1/2 + 1/2)^2$$

Apparently, the chromosomal distribution towards the poles (P), if random, is an expansion of the compound binomial

$$P = (3/4 + 1/4)^n \cdot (1/2 + 1/2)^t$$

in which n is the number of univalents and t the number of trivalents. Quadrivalents were treated as two bivalents. The first part of the formula thus depends on the number of univalents, the second part on the number of trivalents. Table XVI shows the ratios for the simplest cell types.

Using a method similar to that for calculating the expected frequency of laggards (Table XIV), the expected frequency of each polar type was calculated.

Again, for each cell type, the fraction of occurrence of each polar type is multiplied by the frequency of the corresponding cell type (expressed in %). These values, calculated for all possible pole combinations of each of the cell types observed at metaphase I of the hybrid, when added together will give the expected ratio of polar distribution of chromosomes at anaphase I (Table XVII). Quadrivalents, here again, are treated as two bivalents. It should be emphasized that in computing the polar distribution, random lagging is assumed to occur. The calculated polar combinations are thus based on random polar distribution and on lagging of the univalents in the anaphase I. The ratios thus calculated were 0.5 (8) : 2.4 (9) : 7.6 (10) : 17.7 (11) : 27.5 (12) : 25.7 (13) : 13.9 (14) : 4.0 (15) : 0.6 (16). The values in brackets indicate the number of chromosomes at the pole.

Table XVII. Outline of the calculation of the theoretical polar distribution at anaphase I from the observed metaphase I cells.

Cell types IV III II I	%	Number of chromosomes distributed towards the poles 7	8	9	10	11	12	13	14	15	16	17	Total %
14	0.157								0.157				
13 2	0.47							0.264	0.176	0.029			
12 4	3.74						1.183	1.578	0.789	0.175	0.015		
11 6	5.62					1.000	2.000	1.667	0.741	0.185	0.025	0.001	
etc.	etc.								etc.				
Total distribution for cells with:													
no multivalents	23.87	0.030	0.232	0.951	2.427	4.663	6.591	5.568	2.696	0.618	0.082	0.007	23.87
1 trivalent	56.16	0.021	0.217	1.003	3.769	9.701	15.647	14.961	8.083	2.351	0.360	0.029	56.14
2 trivalents	14.20	0.017	0.062	0.243	0.875	2.215	3.663	3.793	2.339	0.811	0.148	0.014	14.19
3 trivalents	1.40		0.003	0.022	0.084	0.206	0.342	0.371	0.248	0.099	0.023	0.003	1.40
1 quadrivalent	1.40				0.031	0.138	0.356	0.528	0.283	0.058	0.004		1.40
1 trivalent + 1 quadrivalent	2.96	0.002	0.010	0.043	0.149	0.373	0.706	0.896	0.598	0.166	0.017		2.96
Total	99.99	0.073	0.543	2.355	7.504	17.650	27.506	25.749	13.873	3.973	0.610	0.050	99.95
Expected pole distribution in Anaphase I			0.5	2.4	7.6	17.7	27.5	25.7	13.9	4.0	0.6		100%

The results of the observation of 144 anaphase I cells can now be compared with this theoretical ratio. The poles with the same chromosome number are added giving a population of 288 cells. The observed ratio then becomes: 2 (18) : 7 (9) : 31 (10) : 43 (11) : 71 (12) : 59 (13) : 22 (14) : 7 (15) : 1 (16) : 1 (17). Values in brackets again indicate the polar distribution. Table XVIII shows the results of the X^2 computations.

Table XVIII. Polar distributions of chromosomes as observed at anaphase I cells compared with the expected values, calculated from the observed metaphase I cells in the hybrid T. dicoccoides x T. timopheevi.

Poles with	Observed anaphase I poles (O)	Expected and calculated pole distribution (T)	$\frac{(O - T)^2}{T}$
8	2	1.4	0.26
9	9	6.9	0.64
10	33	21.9	5.63
11	45	51.0	0.71
12	89	79.2	1.21
13	66	74.0	0.86
14	32	40.0	1.60
15	10	11.5	0.20
16	1	1.7	0.05
17	1*	-	
Total	288		X^2 = 11.16
		DF = 8;	P = 0.19

* This class is not included in the calculation

The X^2 value is below the significant level with a P = 0.19, indicating an acceptable correspondence between observed and theoretical data. The one pole with 17 chromosomes is taken out of consideration, since this pole would have increased the X^2 value tremendously. In the comparison made in Table XVI it appeared that the observed and expected polar distributions were very close in all but two of these classes: those poles with 14 chromosomes, which have a lower frequency than the expected number; and those with 12 and 10 chromosomes, which have, in contrast, a much higher frequency than the expected one. This feature may be directly related to the excess number of laggards also observed in this same population (Table XV). But since the X^2 value for the polar distribution of chromosomes of anaphase I cells is non-significant, the author believes that these phenomena (excess of laggards and of polar distributions with small chromosome numbers) can be attributed to sample size and sample error.

From these calculations it may be assumed that univalent and multivalent behaviour at anaphase I are random. Granting this, the basic assumption of random chromosome behaviour may be accepted as valid.

To verify the assumption that only gametes with normal chromosome complements are viable, the offspring of the hybrid must be investigated. This will be the subject of discussion in the following section.

E. THE OFFSPRING OF THE HYBRIDS

(a) T. timopheevi (1) x T. durum var. leucomelan

Six F_2 plants of the hybrid, T. timopheevi x T. durum var. leucomelan, were grown in the greenhouse in the fall of 1957. The meioses of these plants were studied to determine average bivalent, trivalent and univalent formation. A minimum of 50 cells was considered essential to determine the average frequencies for each type of chromosome association. Table XIX shows the chromosome numbers, the average frequency per chromosome association and the chiasmata formed per bivalent observed in the six F_2 plants. Column 7 gives in simplified form the chromosome associations representative for particular F_2 plant involved. In determining these chromosome associations, the average frequencies and only the most frequently occurring cell types were taken into consideration. Plant no. 2 with 20 chromosomes is treated as having no trivalent, although column 5 indicates an average 0.50 trivalents. However, from a cytology study of this plant it is inferred that the trivalent involves the extra chromosome (probably transmitted through the egg cell). Since column 7 is based on 28 chromosomes per plant, it can be seen that this particular F_2 plant has been treated as having no trivalent.

Table XIX provides several interesting points. Firstly, from the six F_2 plants five had 28 and one had 29 chromosomes. The 29-chromosome plant must have arisen from an egg cell with 15 chromosomes, fertilized by a pollen cell with 14 chromosomes. This further indicates that the basic assumption, concerning the

Table XIX. Meiotic behaviour of the F_2 generation of the cross, T. timopheevi (1) x T. durum var. leucomelan

1	2	3	4	5					6	7		
No. F_2 plants	No. of M I cells studied	Chrom. no. (2n)	Chrom. no. F_1 gamete	Frequency per chromosome association type V	IV	III	II	I	Chiasmata per bivalent	III	II	I
1	140	28	14	-	0.04	1.04	10.41	3.91	1.74	1	10	5
2	90	29	15	-	-	0.50	11.00	6.00	1.70	-	11	6
3	77	28	14	-	0.05	1.08	10.83	2.90	1.63	1	11	3
4	127	28	14	0.01	0.04	0.94	11.08	2.80	1.63	1	11	3
5	73	28	14	-	-	0.07	11.00	5.77	1.66	-	11	6
6	84	28	14	-	0.07	0.15	12.25	2.77	1.58	-	13	2

viability of only the normal 14 chromosome gametes, is not entirely valid, since apparently also gametes with 15 chromosomes are functional.

The second point of interest is the segregation of the F_2 for plants with and without trivalents, apparently in a 1:1 ratio. This behaviour was predicted on the basis of observations in the F_1 hybrids (see pages 52 and 53). The F_2 plants also show a kind of segregation for pairing behaviour, since several plants exhibited different modes among pairing classes. Plant no. 6 showed 13 bivalents and 2 univalents, whereas plant no. 2 and 5 showed 11 bivalents and 6 univalents. This phenomenon was also observed in the offspring of the F_1-hybrid T. dicoccoides x T. timopheevi (2).

The third point of significance is the chiasma formation in the F_2 plants. Column 6 shows the average chiasmata per bivalent for each F_2-plant. In these plants the constancy of this value per cell type was striking. The average values of the F_2 plants were in all cases very representative of all cell types in the respective plants. In the F_1 hybrids of the crosses T. timopheevi x T. durum and T. dicoccoides x T. timopheevi, the average values for chiasmata per bivalent were the same (1.48). In the offspring, however, a great variation from plant to plant was observed (see also Table XX, column 6). This may indicate that the chiasma formation per bivalent is genetically controlled, and that the variation of values in the F_2 is actually a segregation for high and low values of chiasmata per bivalent.

Table XX. Meiotic behaviour of the offspring of the F_1 hybrids, open pollinated, of T. dicoccoides x T. timopheevi (2)

1	2	3	4		5				6	7		
No. of plant	No. of cells studied	Chrom. no. (2n)	Probable chrom. no. of F-gamete	Probable chrom. no. of M-gamete	Frequencies per chromosome association type IV	III	II	I	Chiasmata per bivalent	III	II	I
1-5-1 (F_2)	19	28	14	15		?			-	1	9	7
1-5-3 (F_2)	63	28	14	14	-	0.06	13.57	0.67	1.73	-	14	-
2-2	76	34	14	20	0.01	0.16	11.24	11.95	1.64	-	12	4
4-3	87	34	14	20	0.03	0.24	9.21	14.92	1.50	-	10	8
1-3	63	35	14 or 15	21 or 20	0.02	0.17	11.52	11.37	1.55	-	12	4
3-1*	46	35	14 or 15	21 or 20	-	0.30	12.07	9.87	1.56	-	13	2
4-1	54	35	14 or 15	21 or 20	0.15	0.24	10.09	14.06	1.45	-	11	6
5-2	65	35	14 or 15	21 or 20	-	0.83	10.27	12.88	1.51	1	10	5
5-4a	42	35	14 or 15	21 or 20	0.07	0.66	11.36	10.00	-	1	11	3
5-4b	49	35	14 or 15	21 or 20	0.02	0.18	10.12	14.14	1.54	-	10	8
2-1	93	36	15	21	0.01	0.63	12.12	9.85	1.51	-	13	2
4-2	64	36	15	21	-	0.27	11.09	13.02	1.47	-	11	6
5-1	144	36	15	21	0.01	0.35	9.99	14.93	1.40	-	10	8

* Chromosomes extremely small, difficult to study.

(b) T. dicoccoides x T. timopheevi (2)

Eighteen seeds that originated from open pollination of the hybrids in the field, were sown in the fall of 1957. Thirteen of the resulting plants were analyzed, the data being summarized in Table XX. Two of these plants had a somatic number of 28 chromosomes and are probably F_2 plants, since no tetraploid wheats grew in their immediate surroundings. The remaining eleven plants arose from seeds, which probably originated from pollination by T. vulgare var. Chinese Spring (2n = 24) and its monosomic lines, which were planted all around the hybrid plants concerned. The somatic number of 34 chromosomes probably originated by fertilization of a 14-chromosome egg cell of the hybrid by a pollen grain with 20 chromosomes (from a monosomic plant). The somatic number of 35 chromosomes may have arisen from union of a female gamete with 14 chromosomes and a male gamete with 21 chromosomes. However, the possibility of combination of a 15 chromosome female gamete and a 20 chromosome male gamete also exists. The F_2 plants with 35 chromosomes thus do not indicate clearly the chromosome number of the gametes from which they originated. The three plants with 36 chromosomes came from a 15 chromosome egg cell and a 21 chromosome pollen grain (Table XX, column 4).

These somatic chromosome numbers indicate that hybrid gametes with 15 chromosomes are viable. Calculation of an adjustment for the theoretical fertility value of the hybrid plants now becomes necessary since this value was originally computed on the basis of viability of only those gametes with 14 chromosomes. The calculation will be made after a discussion of the offspring of the hybrids.

The F_2 plants, No. 1-5-1 and No. 1-5-3 showed extremes in meiotic behaviour. The first F_2 plant was meiotically very similar to the F_1 parent. Unfortunately sufficient cells to calculate its average frequency per chromosome association could not be obtained. Still, enough cells were studied to permit the assumption that the value for chromosome associations mentioned in the last column of Table XX is correct. The low bivalent formation in this plant is in striking contrast with the second F_2 plant (1-5-3) which showed an average of almost 14 bivalents per cell. These two extremes, together with the intermediate meiotic behaviour of the F_2 plants described in Table XIX, indicate a definite segregation for bivalent formation.

The remaining plants of this offspring show the same tendencies: plants with high numbers and plants with low numbers of bivalents. Since these plants originated from open-pollination they can actually be considered as backcross progeny because T. aestivum possess the A- and B-genome. By subtracting the D-genome (6 or 7 chromosomes respectively) one may obtain the picture that would occur in an actual backcross generation (column 7). One may dispute the legitimacy of this, since (a) there may exist differences in the B-genomes of the various wheat species; and (b) the genes on the chromosomes of the D-genome may have a definite effect on pairing behaviour. Since the number of F_2 individuals in this offspring is very small, no definite conclusions can be drawn from it until a more extensive investigation is done on a larger F_2 or backcross generation. With the material now available, therefore, one can only search for tendencies without attempting definite conclusions. For such a search of tendencies the

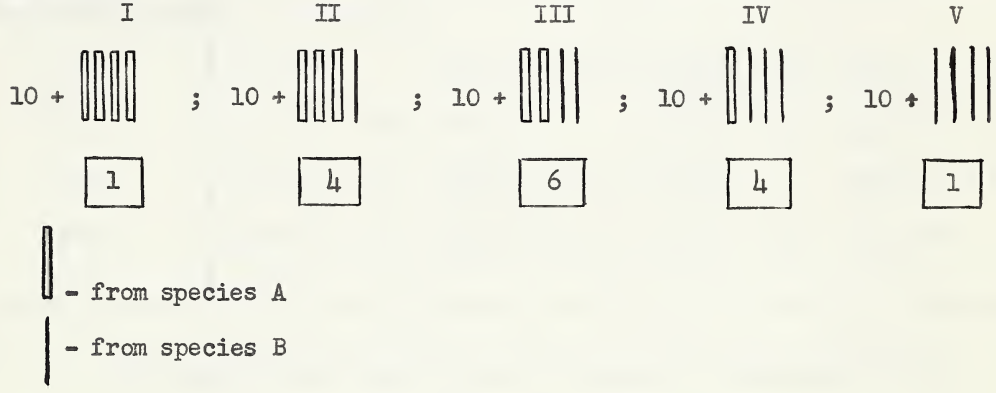
author believes that it is permissable to compare the meiotic behaviour in plants with 34 to 36 chromosomes with the pairing at the tetraploid level (28 chromosomes) in the other F_2 plants. This is done in column 7 of Table XX. The segregation in meiotic behaviour is also very pronounced in this group of plants. There is, however, a preponderance of plants lacking the trivalent association. Only two plants out of the eleven showed trivalents. This may be due to the different B-genomes of T. aestivum. Plants 4-2 and 5-1, although they have one chromosome extra, do not show trivalent formation very frequently. Plant 2-1 fairly often showed cells with a trivalent, but cells without trivalents were almost as frequent. From this it is believed that the trivalent originated from an extra chromosome transmitted through the egg cell.

One may wonder why the meioses of the offspring plants segregate. This can be explained genically or chromosomally. If the irregular meiosis of the F_1 is genically induced, one may find a segregation of this effect in their offspring. The nature of this meiotic segregation will depend on the number of genes involved. The pairing behaviour in these plants shows the extremes (plant 1-5-1 and 1-5-3) although the middle group (with 11 to 13 bivalents) is best represented. If the asynapsis in the F_1 is genically induced, more than one gene must be operating to give this kind of segregation.

The other alternative is that the meiotic behaviour of the offspring is chromosomally induced. We may assume that the irregular pairing of the F_1 hybrids is induced by structural dissimilarities between some chromosomes of the B- and G-genome. Since the F_1 has an average of 10 bivalents and 8 univalents (actually 1 trivalent,

9 bivalents and 7 univalents; the trivalent being split-up into 1 bivalent and 1 univalent, giving 10 bivalents and 8 univalents) there may be 4 pairs of chromosomes, which have structural dissimilarities and are therefore unable, or able only with difficulty, to pair and form bivalents. The gametes formed from such a meiosis in the hybrid may be shown diagrammatically as follows:

Gamete type



The figures in the squares indicate the ratio in which these gametes will occur.

After self-pollination one of these five types of gametes may fertilize an egg-cell having also the constitution of one of the same types. In working out the possibilities one should remember that in gametes with, for example, three chromosomes of species A and one of species B this latter chromosome may be any one of the four. This is also the case for the other types of gametes with chromosomes of mixed origin.

An example is shown in Table XXI of the possibilities arising if gametes with 10 +  chromosomes unite.

Table XXI. Hypothetical bivalent formation in F_2 plants arising from the union of gametes having three chromosomes from species A and one from species B (indicated by a prime).

	F_1 gametes			
No. of chromosomes from bivalents 10 + →	from univalents			
+ ↓				
from univalents	F_2 combinations			
1'234	$(10+4)^{II}$	$(10+2)^{II} + 4^{I}$	$(10+2)^{II} + 4^{I}$	$(10+2)^{II} + 4^{I}$
12'34	$(10+2)^{II} + 4^{I}$	$(10+4)^{II}$ -	$(10+2)^{II} + 4^{I}$	$(10+2)^{II} + 4^{I}$
123'4	$(10+2)^{II} + 4^{I}$	$(10+2)^{II} + 4^{I}$	$(10+4)^{II}$ -	$(10+2)^{II} + 4^{I}$
1234'	$(10+2)^{II} + 4^{I}$	$(10+2)^{II} + 4^{I}$	$(10+2)^{II} + 4^{I}$	$(10+4)^{II}$ -

This table shows that from the 16 possible combinations four plants will show 14^{II} and twelve plants will show 12 bivalents and 4 univalents. If one works this out for all combinations of gametes (Table XXII), it is found that the expected meiotic segregation in the F_2 is 1 $(10^{II}\ 8^{I})$: 4 $(11^{II}\ 6^{I})$: 6 $(12^{II}\ 4^{I})$: 4 $(13^{II}\ 2^{I})$: 1 (14^{II}).

It is noteworthy that this same ratio would be expected in a backcross generation. Then the hybrid gametes I to V will unite with gametes I or V (depending on the species) and the results will be the same as in an F_2 generation.

The tendency toward this kind of segregation can be seen in the 8 F_2 plants, although this population is too small for definite conclusions. Since the plants with 34 to 36 chromosomes, as we have

Table XXII. Calculation of the ratio of meiotic behaviour in the F_2 of a hybrid between species differing structurally in 4 chromosomes and thus having the characteristic meiotic configuration of 10 bivalents and 8 univalents.

Female gametes	I	II					III						IV					V	Total	Ratio in F_2
Male gametes	I-V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I-V		
Possible F_2 combinations																				
14^{II}	1		4						6							4		1	16	1
$13^{II}\ 2^{I}$	4	4		12				12		12				12		4		4	64	4
$12^{II}\ 4^{I}$	6		12		12		6		24		6		12		12			6	96	6
$11^{II}\ 6^{I}$	4			12		4		12		12		4		12				4	64	4
$10^{II}\ 8^{I}$	1				4				6				4					1	16	1

mentioned previously, are not very useful for this same purpose, conclusions concerning the nature of this segregation must wait until a much larger F_2 or backcross population is obtained.

A few speculations, however, may be permissible. Since there is also a segregation in the number of chiasmata per bivalent (independent of that for bivalent formation) two effects, one a genic effect on chiasma formation, the other an induction of asynapsis by structural chromosome dissimilarities, may occur together. If this is true it must be noticeable in the meiotic segregation of the F_2 plants. The genic effect of low chiasma formation will reduce the number of bivalents expected on the basis of random chromosome segregation. This will cause the normal distribution expressed in the ratio 1 : 4 : 6 : 4 : 1 for chromosome segregation to become skewed toward plants with a lower frequency of bivalent associations. This tendency may be noted in the data of the F_2 generation included in the data of the last column of Tables XIX and XX (trivalents treated as one bivalent and one univalent). Since the meiosis of the two F_1 hybrids are very similar and selfing or backcrossing gives an almost identical meiotic segregation these data are combined together in Table XXIII.

Table XXIII. Frequencies of meiotic behaviour of all offspring plants examined from the hybrids T. timopheevi (1) x T. durum var. leucomelan and T. dicoccoides x T. timopheevi (2)

Chromosome associations	Observed frequency	Theoretical frequency
$10^{II}\ 8^{I}$	4	1
$11^{II}\ 6^{I}$	6	4
$12^{II}\ 4^{I}$	5	6
$13^{II}\ 2^{I}$	3	4
14^{II}	1	1
	19	16

From this table the skewness towards the meiotic types with the lower bivalent frequencies is evident. However, it should again be emphasized that this is speculation and that the 28-chromosome F_2 plants may not be comparable with the other offspring plants with 34 to 36 chromosomes. If such skewing occurs in a larger F_2 generation, this phenomenon may indeed be explained by the theory described above.

F. ADJUSTMENT OF THE THEORETICAL FERTILITY VALUE

It is apparent that some individuals among the offspring of the two hybrids of T. timopheevi originated from gametes with 15 chromosomes. These were probably female gametes since the certation-effect would probably give a strong selection in favour of pollen with 14 chromosomes.

This functionability of (female) gametes with 15 chromosomes makes it necessary to adjust the theoretical fertility value. In the adjustment gametes with 15 chromosomes are considered to be functional only if they have one chromosome extra to a normal set of chromosomes.

The calculations are very similar to those described for normal 14-chromosome gametes on pages 62 to 65. The matter is, however, somewhat more complicated, since no general formula could be established for the viable fraction. The cells without trivalents showed a viable fraction of $\frac{(12)^{\frac{1}{2}(n-1)}}{(16)^{\frac{1}{2}n}}$. For the cells with trivalents a fraction of 1/8 was also involved. A formula could not be worked out since the cells with lower numbers of univalents apparently followed a different series. It must, therefore, be pointed out that the calculated adjustment for the theoretical fertility value is only an estimate, although the author believes that this estimated value is very close to its actual value.

The adjustment, calculated for the theoretical fertility value, based on 15 chromosome gametes, proved to be 2.5%. A further

adjustment for multivalents, inducing gametic non-viability (see page 68), brings this value down to approximately 2.2%. The total theoretical fertility value for the hybrid T. dicoccoides x T. timopheevi (2) is then 5.3% + 2.2% = 7.5%. The observed fertility value of the F_1 plant was 4%, giving a discrepancy of 3.5%.

It is desirable to compare the proportions of functional gametes with 15 chromosomes observed in the offspring with the calculated values. Among the offspring, four plants with 36 chromosomes obviously resulting from fertilization by pollen of neighbouring hexaploid wheats, could be definitely identified as having arisen from egg cells with 15 chromosomes. This is approximately 20% of the total population of 19 plants. This value is possibly higher since the number of chromosomes contributed by the egg cell could not be determined with certainty for the plants with 35 chromosomes.

Theoretically the proportion of plants arising from female gametes with 15 chromosomes should be $\frac{2.2}{5.3} \times 100\% = 41.5\%$ of the total number of gametes. Since the observed 20% is considerably lower than this, it appears probable that an appreciable proportion of gametes with a normal complement plus an extra chromosome are non-functional.

G. DISCUSSION

In the preceding sections it appeared that the F_1 hybrids of T. dicoccoides x T. timopheevi (2) and T. timopheevi (1) x T. durum var. leucomelan form a certain amount of viable gametes, able to produce healthy and normal offspring. Viable gametes are produced by these hybrids despite a very irregular meiosis. Further it was observed that gametes with 15 as well as with 14 chromosomes were functional.

Calculations based on observations of metaphase I in pollen mother cells, and on the assumption that the same conditions hold for embryo-sac mother-cells, indicated that 7.5% of the embryo sacs produced by these plants should contain combinations of chromosomes ensuring viability. Furthermore, since these plants produce ample pollen fertilization by normal male gametes as the rule, a similar proportion of the embryo sacs (7.5%) should develop into mature kernels. Observations on the actual plant fertility, however, showed only a 4% seed set, and thus indicated a viability of female gametes of 4%. There is thus an apparent gametic lethality of 7.5% - 4% = 3.5% or about 45% of the total amount of theoretically functional eggs. This sterility of gametes with theoretically functional chromosome combinations is probably due to structural differences between some chromosomes leading to genically unbalanced gametes.

From the point of view of the proportion of theoretically functional gametes that are actually functional (55%), the fertility of the hybrids appears quite high. The greatest sterility effect (approximately 92.5%) came from abnormal chromosome distributions due to the irregular meiosis.

Species which produce hybrids with a relatively good fertility are usually considered to be very closely related. A more regular meiosis than observed in the T. timopheevi hybrids is then usually observed. But the cause of irregular meiosis in a hybrid is not always known. Generally, if a genic effect is not directly obvious, chromosomal dissimilarities causing asynapsis are assumed to be the cause of the abnormal meiosis. Chromosomal effect and divisional effect both operating simultaneously and indistinguishably in the gametes are then regarded as a single source of sterility. Different genomic symbols may then be attached to genomes that are actually closely related. It is, therefore, important in the method of estimating the divisional effect of an irregular meiosis, that the sterility due to the divisional effect can be distinguished from sterility effects from other sources. The basis of this distinction is a comparison of the amount of the theoretically functional gametes produced with the amount of gametes actually functional, as observed by seed set in the hybrid. If no seed set is observed the sterility is 100% and this is probably due to a chromosomal effect caused by extensive genome differences, superimposed on the divisional effect. But if the seed set is high enough to indicate that a certain proportion of the gametes with theoretically functional combinations are actually functional, then conclusions may be drawn according to the strength of the observed fertility.

A hybrid showing cryptic structural hybridity produces a normal meiosis, but the sterility is very high. T. timopheevi produces this kind of hybrids with T. armeniacum (Svetozarova, 1939) and T. dicoccoides var. nudiglumis (Sachs, 1953). Of these hybrids it was

reported that they were completely sterile or produced very little seed. On the basis of the regular meiosis it was indicated that T. timopheevi had genomic similarity with both species.

If we eliminated from the total plant sterility the divisional effect of irregular meiosis in the T. dicoccoides x T. timopheevi hybrids, we must conclude that the F_1 plants have a fertility of 55%, which is higher than that for either of the two cryptic structural hybrids mentioned above. The cause of the irregular meiosis may be due to some structural chromosome dissimilarities as well as a genic desynaptic effect, which may increase the irregularity considerably. If this is true for the T. timopheevi hybrids concerned then bivalent formation cannot be a good index of chromosome homology. The high functionability of the normal gametes (with theoretically functional chromosome combinations) may indicate that such a genic system is operating in these hybrids. It indicates also that the two genomes, B and G, are very closely related, although structural differences exist between some chromosomes, causing the 45% sterility of the normal and theoretically viable gametes. It is, therefore, believed that the genomic formula $AA\beta\beta$ of Kostoff (1936) indicates better the actual genomic relationships than the AAGG formula. Instead of β the author would prefer to use a genomic formula as AAB^tB^t. The symbol B^t (similar symbols are used in Aegilops species; Sears, 1947) indicates not only its close relation to the B-genome but also a structural differentiation of some of the chromosomes that is peculiar to T. timopheevi. It is suggested, however, not to use this symbol at least until the extent of the structural dissimilarities of the chromosomes involved is established.

The author feels that there are several problems involved which must be investigated further. These arise mainly from our lack of knowledge of the pairing behaviour of chromosomes with a certain amount of structural differences. Some of these problems are:

(1) Can structural differences of some chromosomes cause the irregularities observed in the F_1 hybrids? As indicated above, probably some genic desynaptic effect may have strengthened these irregularities.

(2) If this is true, how could the segregation of the F_2 for different meiotic behaviour be explained? In the discussion of this phenomenon (page 86) it was explained that such a segregation could be expected if one assumed non-pairing of 4 pairs of chromosomes in the F_1. This asynapsis must have been independent of a genic effect to show a segregation in the F_2. If so, what then is the effect of the asynaptic gene(s)? Segregation of the F_2 for different values of chiasmata per bivalent indicates such a genic effect.

(3) If the irregular meiosis is due to asynapsis of chromosome pairs, independent of a genic effect, how then could we explain the high functionability of 55% of the "viable gametes"? How "similar" must chromosomes be to give a fairly high fertility together with an irregular meiosis?

Although we are not able at present to give reasonable answers to these problems they are, nevertheless, of great importance to a general understanding of chromosome behaviour in hybrids. No critical genome analysis can be undertaken unless these effects and how they operate are known.

Therefore, the tentative conclusion that the B- and G-genomes are very closely related must be supported by further evidence. A large F_2 population is essential. Further additional calculations on the F_1 gamete formation are necessary to determine how many dissimilar chromosomes would be necessary to induce a gametic sterility of 45%. A calculated estimate of this, together with the results of the investigations on the F_1's of (amphiploid T. timopheevi x Ae. squarrosa) x T. aestivum var. Chinese Spring monosomic lines I - XIV inclusive, would probably indicate the pairing behaviour of specific chromosomes in which structural dissimilarities are suspected, and the resulting effect on hybrid fertility. When these investigations are completed, the author hopes to be able to state the actual genome homologies between the B-genome and the second genome of T. timopheevi.

The method of calculating the divisional effect of an irregular meiosis, as described in this thesis, is, in the author's opinion, a valuable aid in genome analysis. This has already been pointed out above. The effect of an irregular meiosis on the fertility of the plant appears generally to have been underestimated. According to the author's calculations, if a plant were to display regularly 13 bivalents and 2 univalents instead of 14 bivalents its fertility would be reduced to 45% (this includes "normal" gametes with 14 and 15 chromosomes). This is an astonishingly radical effect on the plant fertility, induced by just two univalents. This effect would be more drastic as the number of bivalents decreased.

It will be, therefore, desirable to measure the divisional effect in all hybrids and other plants with irregular meiosis, including autotetraploids. In the latter group, adaptation of this method to their particular meiotic pecularities are, of course, necessary. Moreover, it should be emphasized here that calculations should be made on the basis of all of the different cell types observed at meiosis, and not from the average frequency per chromosome association.

It will be clear that the present method of calculating the divisional effect of an irregular meiosis is only useful in diploid plants and tetraploids. It cannot be employed for plants which show a whole series of aneuploids in their offspring, such as frequently occurs in the higher polyploids. Although adaptation is theoretically possible, too many adjustments would have to be made on the calculated fertility value. Within this limitation the method may prove to be useful in measuring the divisional effect of any irregular meiosis.

H. SUMMARY

1. The hybrids, T. timopheevi (2n = 28) x T. durum var. leucomelan (2n = 28) and T. dicoccoides (2n = 28) x T. timopheevi, showed similar irregularities in the meiosis of the respective hybrids. Both hybrids exhibited average chromosome associations of 0.9 trivalents, 9.0 bivalents and 7.3 univalents.

2. A method for determining the gametic sterility effect of the irregular meiotic division ("divisional effect") in these hybrids was worked out.

3. With the formula $(1/2)^t . (3/8)^{\frac{1}{2}(n-t)}$ the theoretical proportion of normal, functional gametes could be calculated. This, with adjustments for trivalents and quadrivalents and for functional gametes with 15 chromosomes, proved to be 7.5% of the total number of gametes formed by the hybrid.

4. The observed fertility was 4% of the total number of florets.

5. The behaviour of univalents and multivalents at anaphase I proved to be of a random nature.

6. The F_2 generation, and plants derived from open pollination of the F_1 hybrid, showed segregation for meiotic behaviour in regard to (a) average frequencies per chromosome association, (b) trivalent and no-trivalent formation, and (c) number of chiasmata per bivalent.

7. From these data it was concluded that the G-genome of T. timopheevi is very closely related to the B-genome of T. dicoccoides (since

55% of the gametes with theoretically viable chromosome combinations were functional).

8. Some problems for future research were pointed out, and an evaluation of the method for calculating the divisional effect of irregular meioses in hybrids and autopolyploids was given.

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